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THE VIABILITY OF THE NODULE BACTERIA OF LEGUMES OUTSIDE OF THE PLANT III, IV, V[1]

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University of Illinois

Received for publication July 31, 1925

This paper reports the results of a continuation of studies of the nodule bacteria of legumes presented in a previous paper, and has for its purpose the determination of the effects of certain factors upon the life and growth of these organisms in the soil and in artificial media.

PART III—FACTORS INFLUENCING THE GROWTH OF B. RADICICOLA

This investigation was conducted in order to study causes of failure with liquid cultures.

Methods employed

The culture used in the succeeding experiments was *B. radicicola* of garden pea, transferred from pure stock to an 8-ounce culture bottle containing mannite agar media freshly made. The age of the culture at time of using was 20 days.

In order to determine the effect of various temperatures, the refrigerator at 10°C. and the incubator at 25° to 28°C. were chosen for storage purposes.

Platings were made at different intervals as indicated in the experiments. The dilution used for plating was from 1–100 to 1–10,000,000. In order to verify the results of the bacterial count, tests for inoculation were conducted. The inoculation was made at the time of plating. It was on the basis of 1 pint of bacterial infusion per bushel of seed, having approximately 150,000 seeds (garden pea).

Experiment 9—The effect of dilution and storage

Three bottles having vigorous growth of *B. radicicola* of garden pea were mixed together and diluted to 3 pints with sterile tap water. This volume was divided into three equal portions and each portion treated differently. To the first portion nothing was added; the second portion was diluted to 1 quart; and the third to 1 gallon. Each dilution was divided into 4 equal portions and put in Erlenmeyer flasks, 2 provided with cotton plugs and the

[1] Portion of a thesis submitted to the faculty of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Parts I and II were published in the preceding issue of SOIL SCIENCE.

other 2 with rubber stoppers. A flask with rubber stopper and one with cotton plug from each dilution were placed in the refrigerator and the other flasks were placed in the incubator.

After the 94-day plating, 0.4 gm. of mannite sugar dissolved in 5 cc. of sterile distilled water was added to containers A, A′c, B, B′c, C, and C′c.

Experiment 10—The effect of cane and mannite liquid media

Each of four 8-ounce bottles was filled with 80 cc. of sterile liquid media and inoculated with 1 cc. of bacterial infusion containing approximately 54

TABLE 1

The effect of dilution and storage upon the number of B. radicicola of garden pea—Experiment 9

CONTAINERS*	TIME OF STORAGE PRECEDING BACTERIAL COUNT									
	Immediate	5 days	10 days	30 days	42 days	60 days	70 days	94 days	106 days	142 days
	millions	*millions*	*millions*	*millions*	*millions*	*millions*	*millions*	*millions*	*millions*	*millions*
A	19.250	60.00	68	60	33	11.0	8.0	6.00	5.200	
Ac	18.750	92.00	80	84			26.0	21.00	5.500	
A′	17.800		149	143	120	100.0	80.0	92.00	91.000	56
A′c	18.000	169.00	164	161			110.0	65.00	47.000	5
B	9.800	108.00	Many	59	42	13.0	10.0	9.00	4.150	
Bc	8.600	95.00	132	40			20.0	10.00	1.000	
B′	9.600	204.00	133	169	164	100.0	80.0	80.00	23.000	17
B′c	9.800	171.00	120	135			100.0	72 00	30 000	7
C	2.290	29.00	40	23	16	1.1	0.3	0.02	0.010	
Cc	3.000	28.95	42	22			5.0	4.00	2.000	
C′	1.930	59.00	49	44	38	20.0	14.0	17.00	24.000	1
C′c	2.250	44.00	61	58						

* A contained 1 pint, with cotton plug.
B contained 1 quart, with cotton plug.
C contained 1 gallon, with cotton plug.
"Prime" indicates refrigerator for storage.
Small "c" indicates container with rubber stopper.

million bacteria per cubic centimeter. The organisms were incubated at 26°C. and bacterial counts were made at 5, 10, 20, 30, and 40 days. After the fortieth day count, the bacterial infusion from each bottle was diluted to 1 quart and used for inoculation test.

Experiment 11—The effect of oxygen supply upon the growth of B. radicicola in solid and in liquid media

Regular mannite media was prepared, except that in the case of liquid media agar-agar was omitted. Both liquid Erlenmeyer flasks and solid media, 80 cc. of the former and 50 cc. of the latter were kept in 100 cc. After the

media had been sterilized and allowed to cool for a few days, 1 cc. of *B. radicicola* infusion of garden pea was transferred to each flask. Immediately after the transfer, some of the flasks were made air-tight by inserting rubber stoppers and sealing with paraffin. The organisms were incubated at 26°C. and bacterial counts and tests for nodule production were made at 10, 20, and 30 days. For bacterial counts, 1 cc. of bacterial infusion from solid media was diluted to 10 cc. with sterile tap water, and then a sample was taken from that diluted infusion. For the inoculation test in this experiment, 1 cc. of bacterial infusion from either media was used to inoculate 5 sterile seeds.

TABLE 2

The effect of cane and mannite liquid media upon growth of B. radicicola of garden pea—Experiment 10

Bacterial counts

MEDIA	PLATINGS AFTER					INOCULATION TEST 40 DAYS
	5 days	10 days	20 days	30 days	40 days	
	millions	*millions*	*millions*	*millions*	*millions*	
Mannite	36	127	79	112	80	Many nodules
Cane	25	45	32	4	3	Few nodules

TABLE 3

The effect of shaking upon the growth of B. radicicola—Experiment 12

Bacteria count

CONDITIONS	AMOUNT OF MEDIA	INITIAL COUNT	PLATINGS AFTER		
			4 days	24 days	30 days
	cc.		*millions*	*millions*	*millions*
Shaken	(a) 100	380,000	60	210	120
	(b) 200	865,000	30	140	50
Not shaken	(a) 100	1,650,000	65	60	40
	(b) 200	900,000	40	20	20

The counts after 10, 20, and 30 days showed that organisms were totally absent from both the solid and liquid media provided with rubber stoppers, where the dilution was 1–10,000 per cubic centimeter. The organisms grown in similar media but with the flasks plugged with cotton were alive and had multiplied greatly, 243 million for solid and 45 million for liquid being found per cubic centimeter at 10-days growth.

Very few nodules were found as the result of inoculation with the infusion from the sealed flasks containing either kind of media. But very abundant nodule production was obtained from the inoculation with the infusion from flasks with cotton plugs. No difference was observed between the solid and liquid media in regard to nodule production.

The results of this experiment seem to indicate that lack of oxygen may be the cause of the organisms surviving for so short a period. Therefore, the following experiments along this line of attack were undertaken.

Experiment 12—Effect of shaking upon growth of B. radicicola

In this experiment, four 8-ounce bottles were used as containers. Two of these bottles received 100 cc. each, and the other two, 200 cc. each, of the media. After the media had been sterilized and allowed to cool for a few days, the organisms were introduced. The inoculation of this media was on the basis of 1 cc. of the infusion of *B. radicicola* of garden pea to 100 cc. of the media. The organisms were incubated at 26°C. One bottle containing 100 cc. and

TABLE 4

Oxygen as a limiting factor during the growth of B. radicicola of garden pea in mannite liquid media—Experiment 13

CONDITIONS	PLATED AFTER	PLATES	DILUTION	COLONIES IN PLATES	BACTERIA PER CUBIC CENTIMETER	NODULES PRODUCED
	days					
Original infusion.....	Initial	E	1–1 million	15	15 million	Many
Cotton plug.....	4	E	1–1 million	25	25 million	Many
Rubber stopper.....	4	E	1–1 million	2	2 million	Few
Cotton plug.....	7	F	1–10 million	7	70 million	Many
Rubber stopper.....	7	F	1–10 million	1	10 million	Few
Cotton plug.....	11	F	1–10 million	5	50 million	Many
Rubber stopper.....	11	E	1–1 million	19	19 million	Several
Cotton plug.....	15	E	1–1 million	115	115 million	Many
Rubber stopper.....	15	E	1–1 million	25	25 million	Many
Cotton plug.....	20	F	1–10 million	17	170 million	Many
Rubber stopper.....	20	E	1–1 million	14	14 million	Several
Cotton plug.....	23	F	1–10 million	16	160 million	Many
Rubber stopper.....	23	B	1–1,000	5	5,000	Scant
Cotton plug.....	27	F	1–10 million	17	170 million	
Rubber stopper.....	27	C	1–10,000	17	17,000	
Cotton plug.....	34	F	1–10 million	3	30 million	Many
Rubber stopper.....	34	B	1–1,000	5	5,000	Scant

one containing 200 cc. were shaken once a day, whereas the other two were left undisturbed. In order to facilitate the shaking and to introduce more oxygen in the liquid without its touching the cotton plug, the bottle was unplugged, flamed, and the solution was stirred with the flamed glass rod. Platings were made immediately after the solution was inoculated and also at 4, 24, and 30 days. Tests for nodule production were also conducted.

Experiment 13—The effect of oxygen supply upon the growth of B. radicicola

This experiment was conducted with the idea gathered from the results of previous experiments, that oxygen in a limited amount is the factor concerned during the growth of *B. radicicola* of garden pea in mannite liquid media.

In each of fourteen 100-cc. Erlenmeyer flasks, 80 cc. of mannite liquid media was placed. An excess of calcium carbonate was added before sterilization to eliminate the possibility of the production of acidity during the process of growth. The media was sterilized in the usual manner and then cooled before the organisms were transferred into it. Each flask was inoculated with 1 cc. infusion of *B. radicicola* of garden pea. In order to limit the amount of oxygen in the media, some of the flasks were provided with rubber stoppers and sealed air-tight with melted paraffin, whereas other flasks were left with cotton plugs. The organisms were incubated at 26°C. and bacterial counts were made after 4, 7, 11, 15, 20, 23, 27, and 34 days. In order to prevent the introduction of oxygen into the media, separate flasks were used for each count; that is, after a sample for plating was taken, the bacterial infusion was discarded and new infusion was used for the next plating. Test for inoculation was also carried out at every plating.

TABLE 5

The effect upon B. radicicola of calcium carbonate and tricalcium phosphate—Experiment 14

CHEMICALS USED	AMOUNT OF MEDIA	PLATINGS AFTER					
		Initial	5 days	10 days	19 days	25 days	32 days
	cc.	*millions*	*millions*	*millions*	*millions*	*millions*	*millions*
Tricalcium phosphate	(a) 100	1.3	45	90	20	40	44
	(b) 200	1.4	70	90	21	30	33
Calcium carbonate	(a) 100	1.3	60	90	40	110	86
	(b) 200	1.4	70	80	40	100	98

Experiment 14—The effect of calcium carbonate and tricalcium phosphate

Two 8-ounce bottles were filled each with 200 cc. of the media, and 2 with 100 cc. To each of 2 bottles, one containing 100 cc. media and the other 200 cc., $\frac{1}{10}$ gm. of tricalcium phosphate was added, while to each of the other 2 bottles $\frac{1}{10}$ gm. of calcium carbonate was added. These chemicals were added to the liquid media before sterilization. After the media had been sterilized and cooled for a few days, *B. radicicola* organisms were transferred to it; the inoculation of the media was at the rate of 1 cc. of bacterial infusion to 100 cc. of sterile media.

Discussion of results of experiments 9, 10, 12, 13, and 14

In experiment 9 the count after immediately plating showed that each of the containers C, Cc, C′, C′c, which is a dilution of 1 gallon, had approximately one-eighth as many organisms as in each of the containers A, Ac, A′, and A′c, a dilution of 1 pint. Likewise, each of the containers B, Bc, B′, and B′c, of 1-quart dilution, had approximately one-half as many as the pint dilution.

This result shows that the organisms were very well distributed in the liquid, and it also showed that the dilution in regard to the number of organisms in a given volume was approximately correct.

After 5 days storage, the bacterial count varied, irrespective of dilution, as for example the container A, a dilution of 1 pint, had 60 million per cubic centimeter, while the container C, a dilution of 1 gallon, had 29 million per cubic centimeter. If the rate of growth were the same in both cases A should have 232 million per cubic centimeter if C had 29 million per cubic centimeter, or C should have 7.5 million per cubic centimeter if A had 60 million per cubic centimeter. This would also be true in case of dilution B. However, B had 108 million per cubic centimeter against 60 million of A.

After 10 days, A had 68 million organisms per cubic centimeter, B had many millions, and C had 40 million per cubic centimeter. Ac had 84 million per cubic centimeter against 132 million of Bc, while Cc had 40 million as against 84 million of Ac. With respect to temperature, dilution in group A had given the following count: Container A with cotton plug stored in incubator had 68 million organisms per cubic centimeter, Ac with rubber stopper stored in incubator had 80 million per cubic centimeter, A′ with cotton plug stored in refrigerator had 149 million per cubic centimeter, and A′c with rubber stopper had 164 million per cubic centimeter. The same result was also obtained in the group C; however, this was not true in case of group B.

The 30-day plating showed that in all cases, the number of organisms per cubic centimeter had decreased, with the exception of containers B′ which had increased from 133 at 10 days to 169; however, it dropped when compared with 204 million per cubic centimeter at the 5-day plating; and container B′c had results similar to B′. The figures show that in all cases, with the exception of the two containers mentioned above, the maximum amount of growth was reached between the tenth and thirtieth days and from that time the organisms began to die out, but the decrease was more significant in the A group than in the B or C group. Likewise, in all three groups, A, B, C, the organisms in the container stored in the incubator died faster than those in containers stored in the refrigerator. At the end of 142 days storage, the count showed that the total organisms in each case had decreased to approximately one-half the original number.

After the ninety-fourth day plating, as it was thought that a supply of carbohydrate might stimulate growth, mannite, $\frac{1}{4}$ gm. dissolved in 5 cc. of sterile distilled water, was added to each of the containers A, A′c, B, B′c, C, and C′c. Plating was made 12 days after, and the count showed that a considerable decrease had resulted, except in C′, which increased from 17 to 24 million.

The test for inoculation accompanying the immediate plating showed abundant nodule production in the A and B groups, but in the C group, the production was very much less. From the 5-day to the 70-day incubation, the nodules produced in all cases were very abundant, then the C group began to decrease after 94 days.

In order to ascertain that each colony counted in plates was a *B. radicicola* of garden pea, tests in milk, on potato, and for inoculation were conducted on a plate containing 11 colonies, plated from container A'c. A transfer from each colony growing in the plate was made to a tube of mannite agar slant where it was allowed to grow for 2 weeks before testing. In 11 tubes of sterile milk after 16 days there was a growth which was typical of *B. radicicola* of garden pea when grown in milk, as described by Löhnis and Hansen (1). The test in potato also verified the organism as *B. radicicola* of garden pea. When the content of each of the 11 tubes was used for inoculation, nodule production was obtained.

In experiment 10, it was found that mannite media was more favorable for the growth of *B. radicicola* than cane sugar media. However, no material difference was noticed between solid and liquid media. These results were verified by the inoculation test.

The results of experiment 12 show that organisms had multiplied more in the bottles which were shaken than those in the unshaken bottles. The difference was not noticed in the early plating, but at the 24- and the 30-day platings, the shaken bottles of both volumes contained almost four times as many bacteria per cubic centimeter as the unshaken bottles of corresponding volume. It was also noticed that in both cases, shaken or unshaken, the volumes of greater depth had given fewer bacteria per cubic centimeter than the volumes of less depth. This result showed without question that oxygen was the cause of the depression of growth. As in the case of the shaken bottles, oxygen was supplied more or less in proportion to the demand of the organisms, but this method of supplying was not highly efficient in the case of greater depth, because for instance, in the 200 cubic centimeter of media the stirring was not as thorough as in 100 cc. Besides, the rate of diffusion of air was slower in the greater depth than in the lesser depth, because of greater backward pressure of the liquid. In other words, the amounts of oxygen introduced by stirring and by diffusing were not enough either for reproduction or for protection against any toxic products developed as the result of the activity of the organism during the life process.

No difference in nodule production was observed between the two conditions under which the *B. radicicola* of garden pea was grown; this was due to the fact that more than enough organisms were present to produce abundant nodules for the plant.

Results obtained from experiment 13 showed that the organisms with a limited amount of oxygen decreased from 15 million to 2 million per cubic centimeter within 4 days, whereas those with excess oxygen had almost doubled in number in the same length of time. The organisms with excess oxygen continued to increase up to 170 million, whereas those grown in a limited amount of oxygen had decreased very considerably, to as low as 5,000 per cubic centimeter. This result was verified by the nodule production test.

It was found in experiment 14 that calcium carbonate was very much more

favorable for the multiplication of *B. radicicola* of garden pea than was tricalcium phosphate. However, the difference was not noticed until after 19 days growth in the media. The highest number in either 100 cc. or 200 cc. ever reached by the organisms grown in media containing tricalcium phosphate was 90 million per cubic centimeter. This occurred at the 10-day plating, and from that time on, the number began to decrease. But on the other hand, the organisms grown in media containing calcium carbonate reached 110 million and 100 million per cubic centimeter in 100 cc. and 200 cc. respectively. This maximum growth occurred at a later stage of the growing period, 25 days after inoculation. In all cases, either in media containing tricalcium phosphate or calcium carbonate, the 100-cc. volumes gave higher results on the average. This difference, however, did not begin to show up until the 10-day plating. At the 5-day plating, the result was reversed, the 200-cc. volumes having more bacteria per cubic centimeter than the 100-cc. volumes.

PART IV—THERMAL DEATH POINT IN SOIL AND ON ARTIFICIAL MEDIA

Methods employed

The tubes were made as follows with 6 mm. of soft glass tubing: One-third of a 1-foot length, was heated over a blue flame, drawn to a capillary size and bent in U-shape with a capillary stem one-third as long as the larger stem. The ends were sealed by heating them over a blue flame, thereby creating a sterile vacuum inside of the tube. Just before using, the end of the larger stem was cut off with a heated three-cornered file. The cut end was heated over a flame and a cotton plug was inserted while it was hot.

One cubic centimeter of an infusion containing organisms was delivered with a sterile 1-cc. pipette into the tube through its larger stem, by removing the cotton plug, which was returned as soon as possible. The tip of the capillary stem was broken off with heated pincers, thereby allowing the infusion to flow as far down in the tube as desired, when the tip was again sealed. The tube containing the infusion was attached to a standard thermometer immersed in sterile tap water in an Erlenmeyer flask. The water was constantly stirred with a stirring rod in order to keep the temperature uniform throughout the entire volume. The temperature was lowered by the application of ground ice, and raised by the application of a direct flame at the bottom of the flask. In all cases, the immersed tube was held in water for 10 minutes at every temperature, 0, 10, 20, 30, 40, 50, 60, and 70°C. The contents of the tube were removed either by breaking the capillary end or by removing the cotton plug from the larger stem. In case of soil, no special apparatus was used in transferring it to the capillary tube; however, all the necessary precautions were used in order to avoid any possible contaminations.

Experiment 15—Thermal death point of nodule bacteria, B. radiobacter and Azotobacter chroococcum

The results as reported in table 6 show that *Bacillus radiobacter* and *Azotobacter chroococcum* were alive at 50°C. for 10 minutes exposure, but all died at a

60°C. exposure for 10 minutes. *Bacillus radicicola* of garden pea and of sweet closer and *Ps. radicicola* of cowpea were alive at 40°C. for 10 minutes exposure, but all died at a 50°C. exposure for 10 minutes. Zipfel (2) reported that the thermal death point of *B. radicicola* was 60°C. to 62°C. for 5 minutes exposure.

After having found the thermal death point of the organisms, the percentage dead or alive at the highest temperature at which they were found to live was determined. The determination was made in two ways: one was by diluting the treated infusion to the dilutions desired and plating out from them, and the other by diluting the untreated infusion and transferring from each dilution 1 cc. to the capillary tube, where it was subjected to the temperature desired.

The platings made from dilutions of treated infusion of *B. radicicola* of garden pea, showed that after being held at 46°C. for 10 minutes, only approxi-

TABLE 6

Thermal death point of nodule bacteria, B. radiobacter, and Azotobacter chroococcum in infusion—Experiment 15

ORGANISM	AGE OF INFUSION	TIME OF EXPOSURE	TEMPERATURE			
			40°C.	50°C.	60°C.	70°C.
	days	*minutes*				
Garden pea	5	10	+	−	−	−
	20	10	+	−	−	−
Sweet clover	5	10	+	−	−	−
	20	10	+	−	−	−
Cowpea	5	10	+	−	−	−
	20	10	+	−	−	−
B. radiobacter	5	10	+	−	−	−
	20	10	+	+	−	−
Azotobacter	5	10	+	+	−	−
	20	10	+	+	−	−

mately 4.5 per cent out of 100,000,000 bacteria were alive, and at 47°C. for 10 minutes, only approximately 0.04 per cent were alive. The platings made from infusion in the second determination showed that *Bacillus radicicola* all died at 46°C. for 10 minutes exposure.

Experiment 16—The effect of different kinds of soil upon the thermal death point of legume organisms

In this experiment peat and brown silt loam were used. Portions of 10 gm. of each were carefully crushed up to free them from coarse particles and then placed in 40-cc. Erlenmeyer flasks with cotton plugs. After the flasks were sterilized and cooled for a few days, *B. radicicola* infusion of 10 days growth in

mannite agar, was transferred into them. The inoculation of these soils was at the rate of 1 cc. infusion to 10 gm. of sterile soil. The first thermal death point determination on *B. radicicola* in soil was made after 3 days and the second after 6 days. After the second determination, the soils were stored in the laboratory drawer and 6 months later they were used to inoculate sterile

TABLE 7

Number of organisms surviving at temperatures near the thermal death point—Experiment 15

TIME OF IMMERSION	TEMPERATURE	PLATE*	NUMBER OF COLONIES	BACTERIA ALIVE	
minutes	°C.				
Plating from dilution of treated infusion of B. radicicola of garden pea					
10	46	A	Many	Many	4.5 per cent
10	46	B	Many	Many	
10	46	C	50	5 million	
10	46	D	4	4 million	
None	Unheated	A	Many	Many	
None	Unheated	B	Many	Many	
None	Unheated	C	Many	Many	
None	Unheated	D	100	100 million	
10	47	A	Many	Many	0.04 per cent
10	47	B	4	40,000	
10	47	C	None	None	
10	47	D	None	None	
Plating from treated dilution of infusion of B. radicicola of garden pea					
None	Unheated	A	Many	Many	
None	Unheated	B	Many	Many	
None	Unheated	C	Many	Many	
None	Unheated	D	100	100 million	
10	46	A	None		
10	46	B	None		
10	46	C	None		
10	46	D	None		

* Dilution A = 1–1,000.
Dilution B = 1–10,000.
Dilution C = 1–100,000.
Dilution D = 1–1,000,000.

seeds. The inoculation was at the rate of 1 gm. of soil to 14 gm. of seed. In order to determine further whether the organisms in the soils were totally dead, the remaining soils of each kind were used to inoculate 5 seeds. These treated soils were tested for acidity with Richorpoor solution.

Both determinations showed that *B. radicicola* of garden pea were alive in

peat after being subjected to 50°C. for 10 minutes. In the second determination, when the inoculated soils were 6 days old, the organisms in brown silt loam were totally dead. In peat, the number of bacteria decreased very considerably. A test at this time indicated that the brown silt loam was acid whereas the peat was not.

The nodule production test showed that the nodule bacteria of garden pea were alive after seven months in peat and were able to produce nodules, but nodules were absent as the result of inoculation with the brown silt loam. This was noticed in six- and in seven-month inoculations.

TABLE 8

The effect of different kinds of soils upon the thermal death point of B. radicicola of garden pea

SOILS	TIME OF IMMERSION	TEMPERATURE	BACTERIA ALIVE	ACIDITY	TEST FOR NODULE PRODUCTION	
					After 6 months	After 7 months
	minutes	°C.				
First determination (1 gm. soil)						
Original infusion	None	Room	Many	None		
Mannite	10	50	None	None		
Peat	None	Room	114,000,000	None	Abundant nodule production	Several nodules were found
	10	50	450,000	None		
Brown silt loam	None	Room	16,000,000	Acid	None	None
	10	50	None	Acid	None	None
Second determination (1 gm. soil)						
Peat	None	Room	2,000,000	None		
	10	46	800,000			
	10	50	350,000			
Brown silt loam	None	Room	None	Acid		
	10	46	None			
	10	50	None			

Experiment 17—The effect of calcium carbonate when present in soil upon the thermal death point of nodule bacteria

This experiment was conducted with the view of obtaining definite data with regard to soil acidity as affecting the thermal death point of nodule bacteria.

Acid brown silt loam soil was used. To 2000 gm. of soil, 2 gm. of potassium phosphate was added and then thoroughly mixed. This amount was divided into two equal portions to one of which 1 gm. of calcium carbonate was added and thoroughly mixed. Each lot of soil was inoculated with one-half bottle

infusions each of pea, cowpea, soybean, and sweet clover bacteria. After inoculation, the soil was put aside and 9 days later the thermal death point was determined. This soil was then used for inoculation on the basis of 1 gm. of soil to 14 gm. of seed. It was stored in bags and kept in the laboratory, and after 5 months it was used for inoculation without being heated.

In all cases, abundant nodule production was obtained from inoculated soil with carbonate heated to 40°C., and 50°C. for 10 minutes, but no nodules were produced from soil with potassium phosphate and no carbonate. Even with the unlimed inoculated soil which was not subjected to heat, nodules were not

TABLE 9

The effect of calcium carbonate in soil upon the thermal death point of nodule bacteria

Heated soil used for inoculation

TREATMENTS	ORGANISMS	TIME	TEMPERATURE	NUMBER OF PLANTS	PLANTS WITH NODULES	NUMBER OF NODULES
		minutes	°C.			
Lime + inoculated soil.....	Garden pea	None	Room	7	7	Many
Lime + inoculated soil.....	Garden pea	10	40	6	6	Several
Lime + inoculated soil.....	Garden pea	10	50	6	6	Several
Inoculated soil.....	Garden pea	None	Room	6	None	None
Inoculated soil.....	Garden pea	10	50	6	None	None
Lime + inoculated soil.....	Cowpea	None	Room	5	5	Several
Lime + inoculated soil.....	Cowpea	10	40	3	3	Several
Inoculated soil.....	Cowpea	None	Room	2	None	None
Inoculated soil.....	Cowpea	10	40	4	1	1 nodule
Lime + inoculated soil.....	Soybean	None	Room	6	2	Several
Lime + inoculated soil.....	Soybean	10	40	4	4	Several
Lime + inoculated soil.....	Soybean	10	50	6	6	Several
Inoculated soil.....	Soybean	None	Room	6	2	2 nodules
Inoculated soil.....	Soybean	10	40	6	2	2 nodules
Lime + inoculated soil.....	Sweet clover	10	40	6	6	Many
Lime + inoculated soil.....	Sweet clover	10	50	6	6	Many
Inoculated soil.....	Sweet clover...	10	40	6	None	None

found on any plant inoculated, with the exception of cowpea and soybean organisms where one and two nodules were found on each plant. This explains the acid resistance of these two organisms.

The 5-months inoculation test, with treated soil not heated, gave the same result as indicated above.

Experiment 18—The effect of soil acidity upon the infecting power of B. radicicola of garden pea

The first part of this experiment was to determine the effect of acid soil upon nodule production, and the second part was to determine the effect of

neutralizing soil acidity upon the nodule production. Three conditions were established as follows: (*a*) A bacterial infusion of *B. radicicola* of garden pea was applied directly to an acid soil, mixed thoroughly and set aside. The next day the non-inoculated seeds were planted in it. (*b*) A non-acid soil was inoculated with bacterial infusion of *B. radicicola* of garden pea, and applied the next day to an acid soil on the basis of 400 pounds of inoculated soil for each acre. Non-inoculated seeds were planted the next day. (*c*) Sterile seeds were inoculated with the bacterial infusion of *B. radicicola* of garden pea, and the next day these inoculated seeds were planted in an acid soil.

In the second part, the soils were treated with calcium carbonate until the acidity was completely neutralized; no definite amount of carbonate was used. These neutralized soils were moistened to about 25 to 30 per cent moisture and then thoroughly mixed. After 2 days storage, during which the moisture content was not materially reduced, they were used as described under the first part.

The soils used were gray silt loam on tight clay, for the first part, gray silt loam on tight clay and brown silt loam for the second part. Both soils were acid although the brown silt loam was not so acid as the gray.

In order to test the infecting power of the organisms used, inoculated seeds were planted in sterile sand under controlled conditions.

In all cases, pint jars were used, each containing 5 seeds, and the plants were allowed to grow for 25 days before nodule examinations were made.

In the first part nodules were absent on all the plants. Not one of the 38 plants grown showed normal growth and gradually died after 20 days. They were washed for nodule examination, however, before they died. The 6 plants in the second part of the experiment that were inoculated from the same bacterial infusion, and then planted in sterile sand, had plenty of nodules, indicating that the infusion used was a nodule producer. The result of the second part showed that under all conditions, all plants in the treated brown silt loam possessed nodules, whereas the plants in the treated gray silt loam on tight clay produced no nodules. The plants in the brown silt loam also looked healthier.

PART V—THE LIFE CYCLE OF VARIOUS NODULE BACTERIA AS INFLUENCED BY CALCIUM CARBONATE, CALCIUM PHOSPHATE, CERTAIN CHLORIDES AND ACIDS

The purpose of this study was to determine the influence of various chemicals on the forms of nodule bacteria, and to determine whether the chemicals used tend to encourage or to inhibit the development of the infective form of the organisms.

Some suggestive work has already been conducted by Bewley and Hutchinson, Löhnis, Löhnis and Smith, Burrill and Hansen, and Whiting and Hansen on stages in the life cycle of nodule bacteria.

This study is concerned with the influence of calcium carbonate and calcium phosphate on the forms of the organisms. In acid soils the organism comes into direct contact with the acid salts. The second part of this work is so planned as to study whether it is the true acidity, the total acidity, the toxic effect of the salts applied, or the absence of calcium in the form of the carbonate and bicarbonate that is most injurious to the growth of the organisms.

Methods employed

The nodule bacteria used were garden pea, red clover, and soybean. These cultures were kept pure during the progress of the work.

Mannite media was used in all experiments. In all the media prepared, excepting the normal one, carbonate was entirely eliminated; however, calcium was supplied in the form of calcium sulfate. Twenty-five cubic centimeters of the prepared media was placed in each of the 50-cc. Erlenmeyer flasks, and the particular element or compound to be added was computed on that volume in per cent for bases and pH values for acids. The acid was sterilized and added to the sterile media. The calcium carbonate and tricalcium phosphate were added to the media before sterilization. In order to obtain an even distribution of the chemical added, the media were constantly shaken while solidifying. One loopful of bacterial infusion was transferred to each flask and then incubated at 26° to 28°C. At frequent intervals microscopic examinations were made from which detailed drawings of many individual organisms are shown in plate 1.

Considerable difficulty was experienced in preparing the slides for staining. The best procedure found was as follows: A sample from the margin of growth was obtained with the point of a platinum needle. This sample was diluted three times with a drop of sterile water at each dilution, being constantly mixed during the process. After a third dilution, the slime had been satisfactorily separated and a loopful of this diluted sample was spread very thinly on the slide and allowed to dry without the application of any artificial heat, after which, carbol-fuchsin stain of very weak dilution (5 cc. of saturated alcoholic fuchsin solution to 45 cc. of 5 per cent carbolic acid solution) was applied and allowed to remain for one minute. Other stains were tried but unsatisfactory results were obtained. To overcome the difficulty with slime, different organic and inorganic solvents, such as dilute alcohol, ether, acids, and physiological salt solutions, were tried but were found ineffective. The stain was then washed in the usual manner. Micro-photographs and drawings were made of representative stains.

Experiment 19—The effect of calcium carbonate

The organisms were grown in media free from carbonate and also in media containing 0.5 per cent of calcium carbonate. It was observed that garden pea and red clover organisms showed a vigorous growth in carbonated media, but a poor growth in non-carbonated media where they died within a month.

Soybean organisms grew equally well in both media for about a month, then the organisms in the non-carbonated media stopped growing and finally died out.

Microscopic examination showed that garden pea organisms grown in non-carbonated media were large, rod-shaped, possessed of a thick cell wall, and contained two or more very defined bands. The average size was 4.03μ long and 1.20μ wide, and the longest observed was 5.64μ long. Those organisms grown in carbonated media were very small and irregular in shape, the cell wall was very thin, single polar individuals predominated, and the whole body did not stain freely. Branched forms were noticed. The average size was 1.6μ long and 0.8μ wide.

Red clover bacteria grown in non-carbonated media exhibited the same form and characteristics as those of garden pea grown in the same kind of media, with the exception that banded individuals were not found among the red clover organisms, but instead granules throughout the body were observed. The average size was 4.03μ long and 1.2μ wide. The longest observed was 6.44μ and the shortest was 1.61μ. In the carbonated media, the organisms were very small, irregular in shape, highly vacuolated, with a branched form predominating. Polar and granulated bodies were also present, and extremely thin cell wall was observed without exception.

In non-carbonated media, the soybean bacteria were small rods staining polar. The average size was 2.41μ long and 0.4μ wide and the longest was 4.8μ. The organisms grown in carbonated media usually possessed three segments, but it was not uncommon to find five or six segments.

Two forms were observed regularly, one with a small round or oblong body half of which was heavily stained, and the other, long and segmented. The average size of the individuals belonging to the first form was 0.4 to 0.5μ long and 0.3μ wide, and for the other was 5μ long and 0.3μ wide. The longest was 8μ and the shortest was 3.4μ.

When these three kinds of nodule bacteria were grown reciprocally, that is, the organisms in carbonated media were transferred to the non-carbonated and those from the non-carbonated were transferred to carbonated, their form changed in accordance with the description above.

Experiment 20—The effect of tricalcium and acid phosphates

In this experiment, the amount of tricalcium phosphate was the same as that of carbonate. But the amount of acid phosphate applied was computed so as to have an initial acidity of pH 5.2 with 25 cc. media used. This pH was established for all organisms concerned in this experiment.

It was found that garden pea and red clover organisms grown in non-phosphated media were large and very much like those grown in non-carbonated media. Soybean bacteria in non-phosphated media were extremely long but showed no change in diameter. The average size was 6.4μ long and 0.4μ wide. The longest were 10μ and mostly single polar organisms, but twos

were occasionally found. The tricalcium and acid phosphate had approximately the same effect upon the form of garden pea and red clover. Tricalcium media had a tendency to produce a branched form, although with both media the formation of round bodies resulted among garden pea and red clover bacteria. These forms may seem to resemble the shape of the first and second stages proposed by Bewley and Hutchinson. With soybean organisms, the acid phosphate media encouraged the formation of long segmented rods and a few oblong organisms. The tricalcium phosphate brought about the formation among the soybeans of oblong bodies very much enlarged at the poles, constituting over a half of the entire body. Long rods were not found in this experiment.

Experiment 21—The effect of aluminum chloride, and hydrochloric, acetic, nitric, and sulfuric acids

The media were acidified by the different acids mentioned and different pH values were established for each kind of organism; pH 5.2 for garden pea and red clover bacteria, and pH 4.5 for soybean bacteria. The pH values were adjusted colorimetrically.

The results showed that aluminum chloride and hydrochloric acid had a similar effect upon the form of garden pea, red clover, and soybean bacteria. The garden pea organisms were club-shaped with very well defined and heavy banding; the red clover were irregular rod-shaped with thin cell walls, but inclosed in each was a mass of more condensed matter running lengthwise from end to end. Soybean organisms were long and segmented.

Acetic acid had a very striking effect upon the form of nodule bacteria, especially of garden peas and red clover. The organisms were flat with very thin cell walls, and with two or three branches. Three-cornered individuals had three poles; four-cornered individuals had four poles, etc. The width was almost the same as the length. This shape was found commonly. The soybean bacteria here were also long segmented rods with sometimes a branched form. The longest observed was 16μ.

The effect of nitric and sulfuric acids upon the form of garden pea and red clover bacteria was the same as that of aluminum chloride and hydrochloric acid. Segmentation was not very common among the soybean organisms. It was found that the bodies were banded with thread-like substances, interwoven with dots. In general, the organisms were large in case of garden pea and red clover, and were long in case of soybean.

SUMMARY

It was found that the rate of multiplication was greater in high dilution than in low dilution, that is, more bacteria were produced in the gallon dilution than in the pint dilution. It was also noticed that *B. radicicola* of garden pea lived long in solution (142 days).

Oxygen was a limiting factor during the growth of nodule bacteria in solu-

tion. Shaking the bottles or containers in which the organisms were growing helped to introduce some oxygen and gave increased growth.

Calcium carbonate added in media, stimulated growth of *B. radicicola* better than tricalcium phosphate.

The heat resistance of *B. radicicola* of garden pea and sweet clover, and *Ps. radicicola* of cowpea and soybean was lower than the heat resistance of *B. radiobacter* and *Azotobater chroococcum*. The legume organisms were killed at 50°C. for 10 minutes exposure, whereas *B. radiobacter* and *Azotobacter chroococcum* were alive at 50°C. for 10 minutes exposure.

The solution in which the organisms were suspended had an influence on the heat penetration: the denser the solution, the slower the heat penetrated, and the thinner the solution, the faster the heat penetrated.

Peat maintained the life of nodule bacteria at a much higher temperature than brown silt loam. Acid soil decreased the thermal death point of legume organisms, and the organisms died in such soil after a short period of storage. The addition of calcium carbonate increased the thermal death point and the keeping quality of legume organisms.

Abnormal media encouraged changes in the forms of nodule bacteria. The normal bacteria were smaller than the bacteroids.

The absence of phosphate or carbonate in the media resulted in the formation of bacteroids. The presence of tricalcium or acid phosphate in the non-carbonated media, did not stimulate the production of active forms.

Aluminum chloride and hydrochloric acid had like effects on the form of legume bacteria.

The presence of acetic, nitric, and sulfuric acids in media changed the legume bacteria into bacteroids and each of these acids affected the form of the organisms specifically.

REFERENCE

(1) LÖHNIS, F., AND HANSEN, R. 1921 Nodule bacteria on leguminous plants. *In* Jour. Agr. Res., v. 20, p. 543.

(2) ZIPPEL, H. 1912 Beiträge zur Morphologie und Biologia der Knollchenbakterien der Leguminosen. *In* Centbl. Bakt. (etc.), v. 32, p. 97–137.

PLATE I

Enlarged from actual size. Magnification × 950. (Carbol-fuchsin stain.)

FIGS. 1, 2 AND 3. Grown in media without calcium carbonate.
FIGS. 4, 5 AND 6. Grown in media with calcium carbonate.
FIGS. 7, 8 AND 9. Grown in media without phosphorus.
FIGS. 10, 11 AND 12. Grown in media with acid phosphate.
FIGS. 13, 14 AND 15. Grown in media with tricalcium phosphate.
FIGS. 16, 17 AND 18. Grown in media with aluminum chloride.
FIGS. 19, 20 AND 21. Grown in media with hydrochloric acid.
FIGS. 22, 23 AND 24. Grown in media with acetic acid.
FIGS. 25, 26 AND 27. Grown in media with nitric acid.
FIGS. 28, 29 AND 30. Grown in media with sulfuric acid.

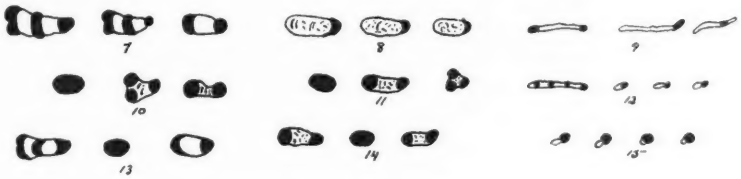
Experiment – I

Effect of Calcium Carbonate

Garden Pea | Red Clover | Soybean

Experiment – II

Effect of Tri-calcium Phosphate and Acid Phosphate



Experiment – III

Effect of Aluminium Chloride, Hydrochloric Acid, Acetic, Nitric, and Sulfuric Acids

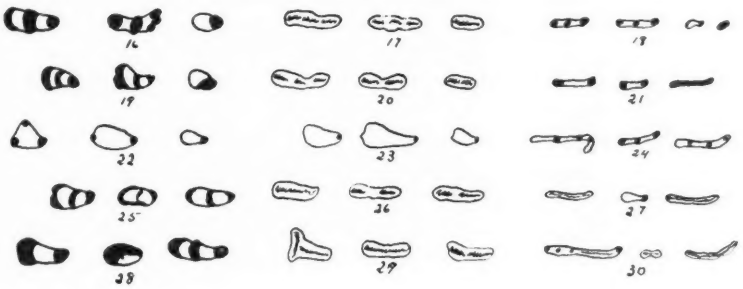
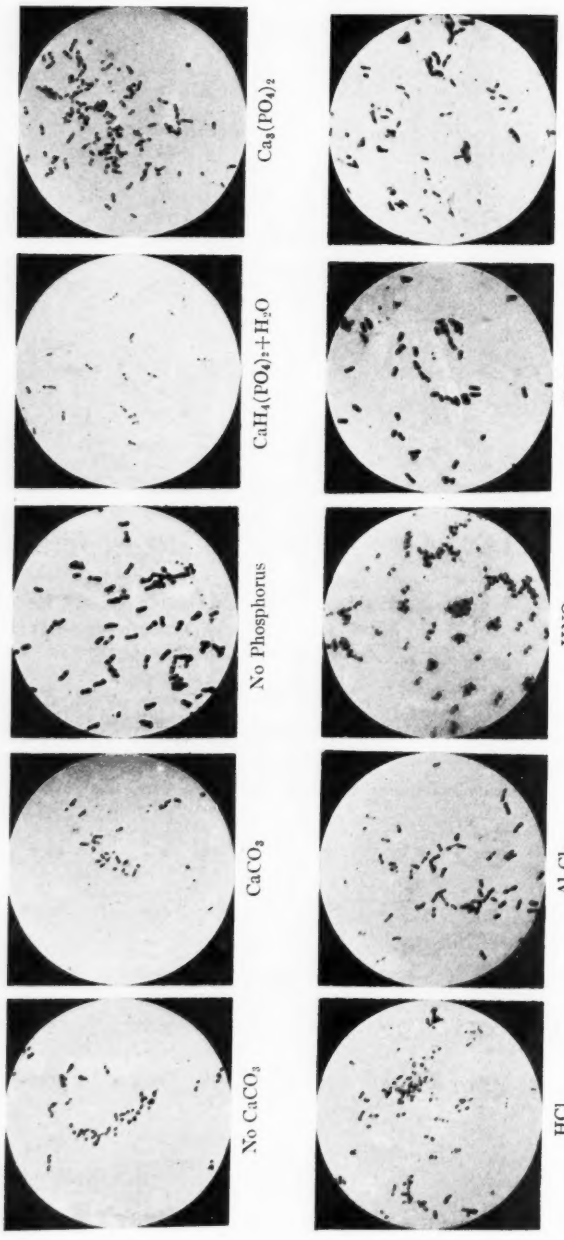


PLATE 2
GARDEN PEA
Stained with carbol-fuchsin. Magnification × 950



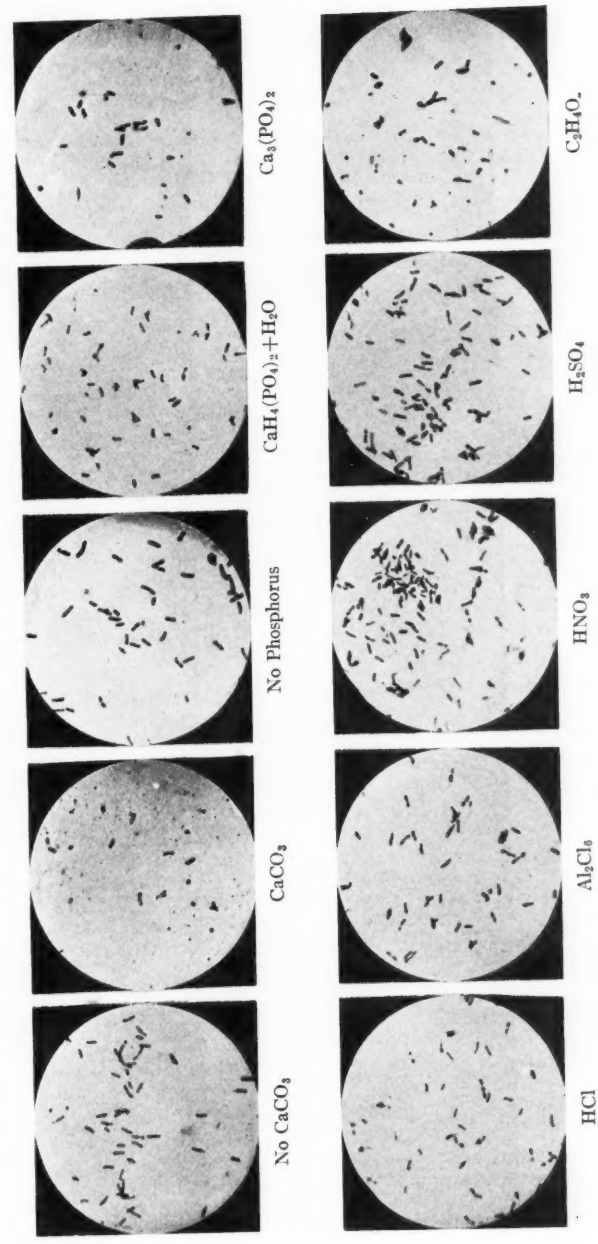
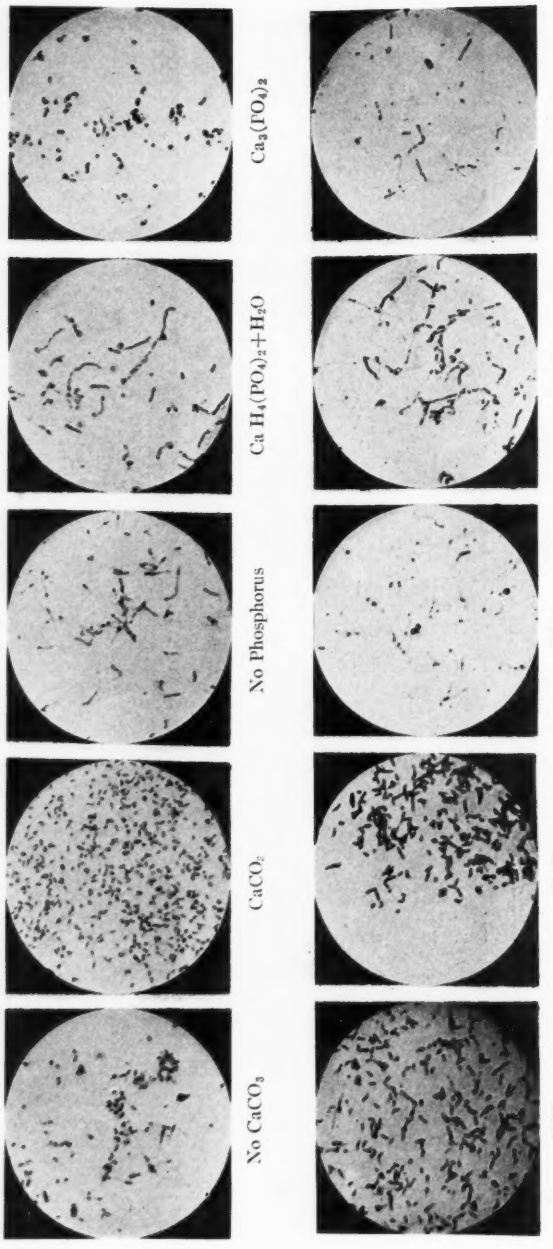


PLATE 3
RED CLOVER
Stained with carbol-fuchsin. Magnification × 950

PLATE 4
SOYBEAN
Stained with carbol-fuchsin. Magnification × 950



THE INFLUENCE OF AVAILABLE NITROGEN ON THE FERMENTATION OF CELLULOSE IN THE SOIL[1]

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PART I

Introduction

The presence of nitrogenous compounds which can be utilized by plants usually determines more than any other factor the success of a crop. Any conditions, therefore, which deal with available nitrogenous compounds and the processes by which they are produced or rendered unavailable are of great practical importance. That the addition of organic material of high carbohydrate content, such as plant residues, to soils is accompanied by a reduction of the available soil nitrogen—nitrates in particular—has been known since the days of Mulder (12). Various circumstances surrounding this loss of nitrates and the process of nitrate formation have long been subjects of research, but until a suitable method for determining cellulose in soils was developed it was quite impossible to determine in a quantitative way the influence of nitrogen on the cellulose, or carbohydrate, decomposition, as only the nitrogen could be determined, which gave us but a partial insight into the biological processes concerned. It is thought that the experiments herein described will be an aid to the understanding of the interrelation between the two processes of carbohydrate decomposition and of nitrate formation.

A review of the literature dealing with the influence of organic compounds on the loss of nitrates in the soil is unnecessary here. It is desirable, however, to summarize the chief deductions from certain researches that are intimately connected with the results obtained from the experiments described in this paper.

In a paper published in 1920 Charpentier (4) described a method for the quantitative determination of cellulose in soils, and in a later paper (5) called attention to the increased rate of cellulose decomposition where manure had been added to the soil. Barthel and Bengtsson

[1] This paper has been published in Swedish as a bulletin from the Central Swedish Agricultural Experiment Station, but because of the inaccessibility of the original to most of the workers in English speaking countries, its English translation is presented in SOIL SCIENCE.

[2] The author wishes to thank Professor Chr. Barthel and Assistants Bengtsson, Sandberg, and Bjälfve for their splendid coöperation and kindness during the period he studied as a Fellow in the former's laboratory.

(2) improved on the method which Charpentier had worked out in the former's laboratory. They studied the influence of ammonium salts, of nitrates, and of stable manure on the course of the cellulose fermentation and showed that the increased rate of fermentation which Charpentier had observed was due to the available nitrogen present in the manure, and not to the bacteria, as sterilized and unsterilized manures of like ammonia content were equally efficient in bringing about this decomposition. In papers published subsequent to the commencement of the experiments herein described, Fred and Viljoen (8), Söderbaum and Barthel (13), Waksman and Heukelekian (15), and Waksman and Starkey (16) have also drawn attention to certain relations existing between the presence of cellulose in the soil and its fertility as influenced by its available nitrogen content. Fred and Viljoen by nitrification and pot experiments studied the cause of poor crop yields on newly cleared land where much undergrowth had been plowed under in the clearing process. They came to the conclusion that the poor yields were caused by a lack of nitrates in the soil due to the cellulose decomposition, and not to toxic materials in the plants plowed under. Söderbaum and Barthel in a series of nitrification and pot experiments with oats showed that the addition of sawdust to soil caused a loss of nitrates during the cellulose fermentation and a corresponding inhibition of vegetation. These conditions continued until the cellulose was destroyed and nitrates were again available for the plants. They found that by the addition of sufficient amounts of sodium nitrate to soil containing sawdust the injurious effects accompanying the cellulose fermentation could be overcome. Waksman and Heukelekian studied the rate of cellulose decomposition in different soils with and without the addition of nitrate nitrogen and determined the number of fungi, bacteria, and actinomycetes present. They found that the number of organisms increased very greatly during the cellulose fermentation, and concluded that the decomposition of cellulose can be added to the group of methods used for carrying out a microbiological analysis of a soil. Waksman and Starkey determined the effect of various organic materials on the numbers of microörganisms in the soil. They found that dextrose, cellulose, rye straw, alfalfa meal, and dried blood greatly increased the number of microorganisms, but that certain substances favored the increase in number of a certain group, or groups, of microörganisms and that other substances increased the number in all groups.

Methods

In these experiments 1 or 1½ per cent of cellulose in the form of very finely divided Munktell filter paper number 10 was added to 200 gm. of heavy clay loam soil. After a thorough mixing the soil was placed in wide-mouthed glass bottles. The various amounts of nitrogen employed were added in the form of an ammonium sulfate or sodium nitrate solution, the same solution being used in experiments 1 and 2. Water was then added to bring the moisture content to its optimum, or 18 per cent. The flasks, after a thorough mixing, which was deferred until the following day for the purpose of obtaining a good soil structure, were stoppered with paraffined cork stoppers provided with short glass tubes containing cotton plugs. The latter permitted a certain amount of aeration with very little change of moisture content. After the desired period of incubation at 18°C., analyses were made for cellulose, for nitrates, and in some instances, for ammonia.

The cellulose determinations were made according to the method worked out in this laboratory by Charpentier and subsequently modified somewhat by Barthel and Bengtsson (2).

The nitrate determinations were made by the usual phenol disulfonic acid method.

Ammonia was determined in experiment 2 by extracting it from the soil with the aid of a potassium chloride solution, as suggested by Bengtsson (3) and Harper (10). The solution was then made alkaline and the ammonia distilled into tenth normal sulfuric acid and determined by Nesslerization, a colorimeter as recommended by Folin (6, 7), being used.

All values, with the exception of a few nitrate and ammonia values, are the average of two closely agreeing analyses, the two results being from different bottles containing soils that received the same treatment, and are not duplicate analyses of the contents of one bottle.

TABLE 1

The rate of cellulose fermentation and accompanying denitrification in the presence of increasing amounts of nitrogen

SAMPLE	NITROGEN ADDED	CELLULOSE FERMENTED IN			NITRATE NITROGEN*			AMMONIA* 42 days	REACTION 42 days
		17 days	24 days	42 days	Start	24 days	42 days		
	mgm.	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*pH*
Check	0	20.7	20.3	18.7	8.6	0	0	0	7.4
1	3.9	41.0	42.0	45.9	8.6	0	0	0	7.1
2	7.8	54.0	59.6	63.1	8.6	0	0	0	6.8
3	11.7	63.0	70.5	81.3	8.6	0	0	0	6.4
4	15.6	68.1	83.9	87.0	8.6	0	Trace	0	6.3
5	19.5	71.4	84.0	90.0	8.6	0	8.3	Trace	6.3
6	23.4	76.2	91.3	92.9	8.6	8.4	23.0	+	6.2
7	27.3	74.2	84.5	92.9	8.6	17.3	41.1	++	6.2
8	31.2	70.1	87.9	92.4	8.6	18.6	96.4	+++	6.1
9	35.1	73.7	87.7	96.8	8.6	21.9	105.0	++++	6.0
10	39.0	74.3	85.3	92.5	8.6	23.0	95.2	+++++	6.0

* per kgm. dry soil.

Experimental

In experiment 1 ammonium sulfate was added to 200 gm. of soil in quantities increasing from one to ten times the amount of ammonia added in a 2 per cent application of stable manure. The ammonia nitrogen present in a certain well decomposed manure was found to be 0.194 per cent. This value was used in calculating the quantities of ammonium sulfate to be added. The control bottles contained the same amounts of soil, cellulose, and moisture, but received no ammonium sulfate, the only nitrogen present being that in the original soil. Analyses for cellulose were made after the bottles had been standing 17, 24, and 42 days at 18°C. Nitrates were determined after 24 and 42 days. The results are shown in table 1, in which the cellulose fermented is given in per cent of the cellulose added. The data are expressed graphically in figure 1, the lower curve indicating the ratio between nitrogen and cellulose fermented after 24 days.

After the first analyses had been made in experiment 1 it was noted that the

sulfuric acid formed by the removal of ammonia from the ammonium sulfate was sufficient to alter considerably the reactions of the soils in the various bottles. Since the fermentation of cellulose is a process which may be participated in by different groups of organisms, such as bacteria, actinomycetes, and molds, it was desired that all soils should be at the same hydrogen-ion concentration so that the fermentations in the different bottles would be brought about by the same groups of organisms. In experiment 1 it was noted that the mold growth increased with increasing amounts of ammonium sulfate and with the accompanying increase of acidity. Experiment 2 was therefore

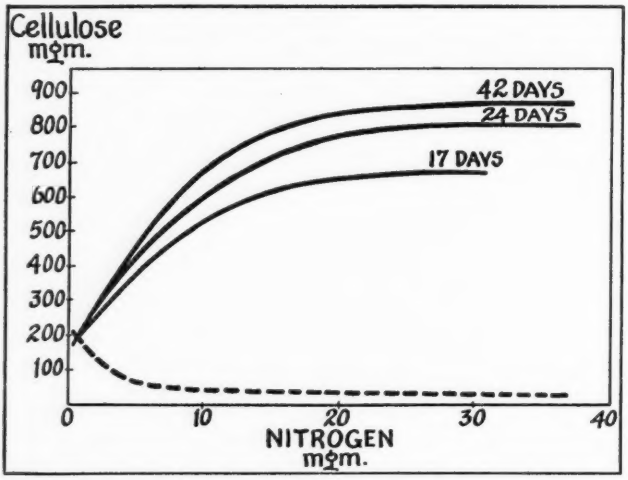

FIG. 1. THE RATE OF CELLULOSE FERMENTATION IN THE PRESENCE OF INCREASING AMOUNTS OF NITROGEN

The three upper curves indicate the amounts of cellulose fermented at various nitrogen after 17, 24, and 42 days respectively. The lower curve shows the ratio of nitrogen to cellulose fermented after 24 days.

conducted with conditions much the same as in experiment 1, but instead of 200 gm. of soil, 198 gm. of soil and 2 gm. of calcium carbonate were used, the latter maintaining a constant reaction of pH 7.3. The amount of cellulose was increased to 1½ per cent. The results of experiment 2 are given in table 2 and are shown graphically in figure 2. In this experiment as in the previous one, the mold content increased with increasing amounts of ammonium sulfate, as might be expected, although the reaction remained constant.

The results of these two experiments indicate that the fermentation of cellulose proceeds with great rapidity in the presence of ammonia or of nitrate nitrogen, the rate of decomposition being dependent upon the relative concentrations of nitrogen and cellulose. The amount of cellulose decomposed per milligram of nitrogen is not constant but varies inversely with the available

nitrogen present in the soil. The ratios of nitrogen utilized to cellulose fermented, as found in the experiments herein described, are given in table 3,

TABLE 2

*The rate of cellulose fermentation and accompanying denitrification in the presence of increasing amounts of nitrogen**

SAMPLE	NITROGEN ADDED	CELLULOSE FERMENTED IN			NITRATE NITROGEN†					AMMONIA NITROGEN†	
		10 days	30 days	60 days	Start	10 days	30 days	50 days	60 days	10 days	30 days
	mgm.	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Control	0	14.6	18.8	22.3	13.8	0	0	0	0		12.3
1	3.9	23.7	37.4	52.0	13.8	0	0	0	0	9.9	....
2	7.8	30.9	49.5	65.0	13.8	0	0	0	0	10.7	13.3
3	11.7	37.7	57.3	74.8	13.8	0	0	0	0	12.5	....
4	15.6	35.9	65.9	87.5	13.8	Trace	0	0	0	15.2	21.8
5	19.5	33.1	64.4	88.7	13.8	8.5	0	0	0	14.2	11.9
6	23.4	34.9	78.5	92.7	13.8	15.3	0	0	Trace	16.0	12.9
7	27.3	33.5	75.9	94.7	13.8	21.2	0	0	8.4	19.6	18.2
8	31.2	32.8	78.2	95.6	13.8	23.4	1.4	13.3	37.8	19.9	21.6
9	35.1	36.3	81.2	96.7	13.8	19.9	8.8	53.6	71.1	33.4	29.7
10	39.0	39.0	83.9	96.6	13.8	14.8	9.9	90.7	115.6	37.9	34.2

*pH=7.3.
†per kgm. dry soil.

TABLE 3

Ratios of nitrogen utilized to cellulose fermented

SAMPLE NUMBER	NITROGEN ADDED	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3	
								After 14 days	
		17 days	24 days	42 days	10 days	30 days	60 days	Ammonia nitrogen	Nitrate nitrogen
	mgm.								
Control	0*	1:222	1:218	1:200	1:145	1:241	1:223	1:72	1:72
1	3.9	1:79	1:81	1:84	1:62	1:98	1:136		
2	7.8	1:58	1:64	1:63	1:47	1:75	1:98		
3	11.7	1:46	1:52	1:60	1:40	1:61	1:79		
4	15.6	1:38	1:50	1:49	1:29	1:54	1:74		
5	19.5	1:32	1:38	1:46	1:23	1:43	1:59	1:14	1:22
6	23.4	1:29	1:34	1:37		1:44	1:52		
7	27.3	1:23	1:30	1:33		1:37			
8	31.2		1:27	1:28		1:30			
9	35.1		1:24	1:26		1:31			
10	39.0		1:22	1:23		1:29			

*The soil used for experiment 1 contained 0.86 mgm.; for experiment 2—1.38 mgm.; and for experiment 3—0.35 mgm. nitrate nitrogen per 100 gm. dry soil.

and are shown graphically in the lower curves of figures 1 and 2. From these it is seen that when the amounts of available nitrogen are very small the ratios of nitrogen utilized to cellulose fermented may be very low, as is indicated by

the control bottles where the only nitrogen present is for the most part in the form of nitrates in the original soil. In experiment 1 where 0.86 mgm. of nitrate nitrogen were present for each hundred grams of soil in the control bottles, 188 and 186 mgm. of cellulose were fermented within 24 days, with corresponding ratios of nitrogen to cellulose of 1:219 and 1:216 respectively. These ratios, however, may not be exactly comparable to those from the soils receiving ammonia nitrogen, for the ratio of nitrogen utilized to cellulose fermented appears to be somewhat higher with nitrate nitrogen than with am-

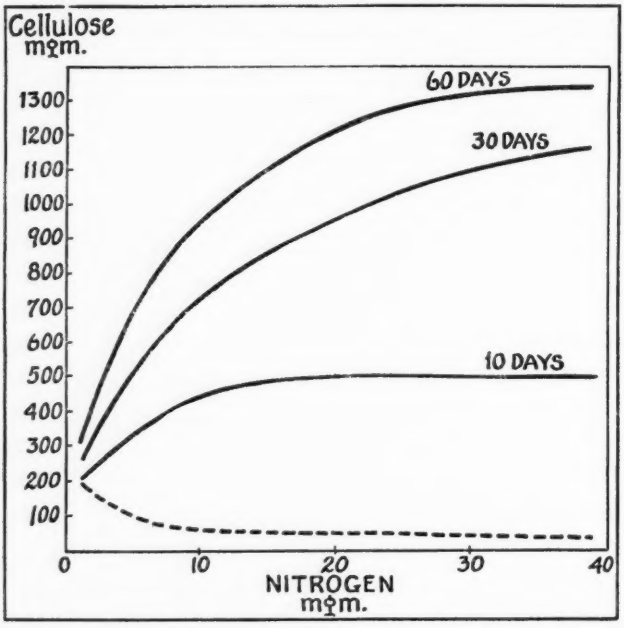

FIG. 2. THE RATE OF CELLULOSE FERMENTATION IN THE PRESENCE OF INCREASING AMOUNTS OF NITROGEN

The three upper curves indicate the amounts of cellulose fermented at various nitrogen concentrations after 10, 30, and 60 days. The lower curve shows the ratio of nitrogen utilized to cellulose fermented after 30 days.

monia nitrogen, as is shown in experiment 3. With increases in the amounts of nitrogen this ratio increased until it reached a value of 1:22 in the soils receiving ten times the amount of ammonia present in a 2 per cent application of manure. Although it is doubtless true that available nitrogen was formed by ammonification and nitrification of the original constitutent of the soil, this amount is almost negligible when compared with the amounts of nitrogen added to the soils, for under conditions similar to these experiments only 14.8 to 15.1 mgm. of nitrate nitrogen was formed per kilogram of dry soil during

three months, or 0.5 mgm. per 100 gm. of soil during each month. This data therefore fails to support the general statement of Waksman and Heukelekian (15, 14) that "for every milligram of nitrogen that is available in the soil or that can become available in a given period, 40 to 50 mgm. of cellulose will be decomposed." This conclusion was doubtless correct for the specific conditions of their experiments.

That nitrification can occur in the presence of appreciable amounts of cellulose is concluded from these results, for in both experiments an accumulation of nitrates occurred when the ratio of nitrogen to cellulose was approximately 1:35. Figure 3 shows graphically the cellulose fermented and the nitrates present from time to time in soil 8, experiment 1, and soil 10, experiment 2. The data of experiment 1 show clearly that nitrates accumulated from the very start of the experiment in those soils which received the largest amounts of ammonia. Where less ammonia was added, all available nitrogen was utilized by the organisms that fermented the cellulose. Under such conditions nitrates could not accumulate. The results of experiment 2 demonstrate this even better, for a more careful study of the nitrification process was made in this experiment. The data do not coincide exactly with those of the former experiment because only 1 per cent of cellulose was used in experiment 1 whereas 1½ per cent was used in experiment 2. It seems clear, therefore, that nitrification occurs in the soil in the presence of cellulose, but that unless the available nitrogen is in excess of that required by the organisms which develop on the cellulose for their nitrogen metabolism, the nitrates will be utilized by the cellulose fermenters as rapidly as they are formed by the nitrate-forming organisms. This is contrary to the opinion expressed by van Iterson (11) and more recently by Gibbs and Werkman (9), that the cellulose inhibits nitrification.

The presence of ammonia is taken to indicate that ammonification can proceed in the presence of considerable amounts of cellulose and that it is not quite so readily utilized by cellulose fermenters as are nitrates, for ammonia was found when nitrates were not.

Experiment 3 was then conducted to determine whether ammonia could be used as such in the fermentation of cellulose or whether it was converted to nitrates and then utilized. For this purpose the soil was acidified with sulfuric acid to a pH of 4.9, a reaction slightly below that at which nitrification is inhibited in this soil. After the soil had been left standing several days to make sure an equilibrium existed between soil and acid the tests were prepared as follows:

Control bottles:	200 gm. soil, 3 gm. cellulose.
Bottles 1a and 1b:	200 gm. soil, 3 gm. cellulose, 23.4 mgm. nitrogen as ammonium sulfate.
Bottles 2a and 2b:	200 gm. soil, 3 gm. cellulose, 23.4 mgm. nitrogen as sodium nitrate.

All bottles were made up to a moisture content of 18 per cent and incubated at 20°C. Analyses were made after 14 and 98 days. The results of this experiment, which are shown in table 4, indicate that in an acid reaction the fermentation of cellulose proceeds with rapidity in the presence of ammonium

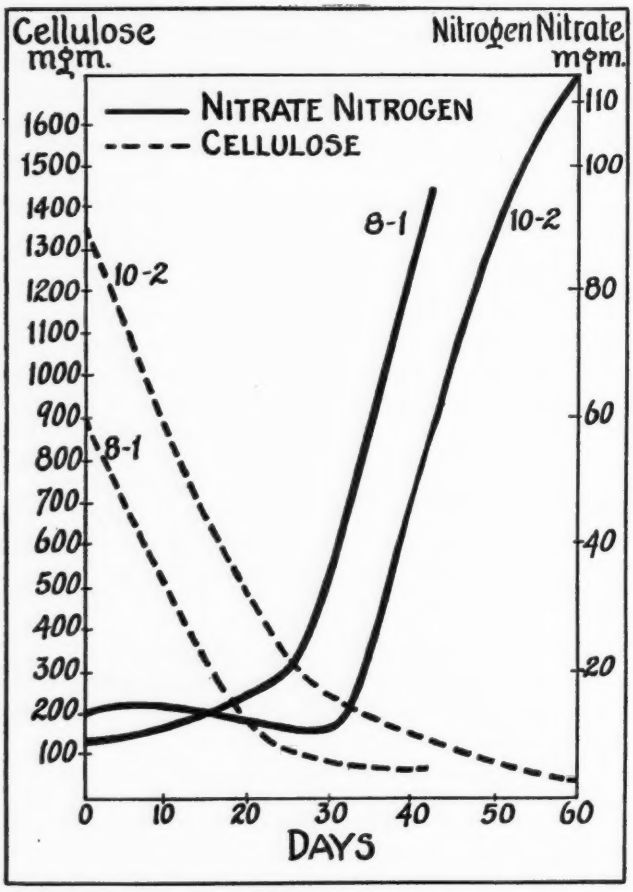

FIG. 3. THE RATES OF CELLULOSE FERMENTATION AND ACCOMPANYING NITRIFICATION
— — — — Cellulose.—8 — 1 = Soil 8, Experiment 1.
———— Nitrate Nitrogen.—10 — 2 = Soil 10, Experiment 2.

nitrogen, and that its conversion to nitrates is not necessary. It is noteworthy that the fermentation proceeded much less rapidly at pH 4.9 than at pH 7.3. The data of experiment 3 and the analyses of the control bottles in experiments 1 and 2 seem to indicate that the presence of nitrate nitrogen accelerates

the cellulose fermentation to a greater extent than does the presence of ammonia. The evidence, however, is rather insufficient to establish such a conclusion as a fact. Ammonia is known to stimulate the development of molds whereas nitrates do not. It may be that in the presence of the nitrates a greater bacterial development occurred of a kind which ferments cellulose more rapidly than do the organisms which were favored by the presence of ammonia. Also, a change of reaction occurred in the two soils as the nitrogen was utilized;

TABLE 4

*The relative availability of ammonia and nitrate nitrogen**

SAMPLE	NITROGEN ADDED		NITRATES AT START	CELLULOSE FERMENTED	
	Form	Amount		14 days	98 days
		mgm.	*mgm.*	*per cent*	*per cent*
Control		0	3.5	11.6	54.0
Control		0	3.5	6.5	
1a	$(NH_4)_2SO_4$	23.4	3.5	23.3	93.4
1b	$(NH_4)_2SO_4$	23.4	3.5	24.0	93.1
2a	$NaNO_3$	23.4	3.5	37.0	100.0
2b	$NaNO_3$	23.4	3.5	38.1	100.0

*pH = 4.9.

TABLE 5

Availability of nitrogenous organic compounds

COMPOUND ADDED TO SOIL	AMMONIA NITROGEN	AMIDE NITROGEN	AMINO NITROGEN	TOTAL NITROGEN	CELLULOSE FERMENTED IN 91 DAYS
$(NH_4)_2SO_4$	3.9			3.9	61.9
Urea		3.90		3.9	60.2
Aspartic acid			3.90	3.9	61.2
Asparagin		1.95	1.95	3.9	59.8
Alanine			3.90	3.9	61.3
Tyrosine			3.90	3.9	55.8
Peptone			3.90	20.5	93.4
Casein				3.9	65.3
Manure	3.9			12.2	67.2
Control				0	25.5
Control				0	28.5

the soil which received the ammonium sulfate becoming more acid, and that which received the sodium nitrate becoming less acid as the fermentation proceeded.

Nitrification did not occur during the above experiment. A control bottle to which ammonium sulfate but no cellulose had been added, contained at the start 17.6 mgm. of nitrate nitrogen per kilogram of dry soil, whereas 16.7 mgm. was present after 20 days. After 98 days 1.3 mgm. of nitrate nitrogen was found in the soils which received sodium nitrate, but none was present in

the bottles which received ammonium sulfate. The presence of small amounts of nitrate is probably due to the neutralization of a part of the soil acidity by the sodium, causing a reaction just within the limit where nitrification occurred.

It may be of interest to describe an experiment to determine the degree in which molds could participate in cellulose fermentation. Five grams of cellulose were placed in each of two bottles. To one bottle was added 10 cc. of the sodium nitrate solution—containing 39 mgm. of nitrate nitrogen—used in the preceding experiment, and to the other was added 10 cc. of the ammonium sulfate solution containing the same amount of nitrogen. Both bottles were inoculated with 0.5 gm. of soil and incubated at 40°C. Within 3 days the bottle to which ammonium sulfate had been added showed a very heavy mold growth, and within 50 days all of the cellulose in this bottle had been completely fermented. Microscopic preparations failed to indicate the presence of bacteria. No visible fermentation of cellulose occurred in the bottle to which the nitrate had been added. It is very likely that at the temperatures under which the experiments were conducted the fermentation due to molds is much greater than occurs under natural soil conditions.

The practical applications of these experiments are numerous, interesting illustrations of the deleterious influence of cellulose in newly cleared land having been cited in the work of Fred and Viljoen (8), of Söderbaum and Barthel (13), and of Gibbs and Werkman (9). Another particularly interesting example is given by Arrhenius (1) in the case of a market gardener who in preparing his forcing beds used heavy applications of shavings which had been used for stable bedding. The cellulose in this bedding brought about a lack of nitrogen in the soil as it underwent fermentation and the young plants were rapidly dying of nitrogen starvation when the attention of the botany department of the experiment station was called to the situation. The crop was saved by the application of sodium nitrate to the soil.

Summary

Cellulose is decomposed in the soil at a rate which increases with the increase in available nitrogen until the latter is present in amounts necessary for the maximum growth of microörganisms. For the conditions of the experiments described in this paper, this point was reached when the ratio of nitrogen to cellulose was about 1:35. When the nitrogen is present in amounts in excess of this ratio it seems to have little influence on the course of the fermentation.

The amounts of cellulose fermented per milligram of nitrogen utilized are not constant but decrease rapidly as the nitrogen increases; in other words, the bacteria are most efficient when their nitrogen supply is limited.

Nitrification can proceed in the presence of cellulose, but unless nitrogen is present in quantities above that required by the bacteria the nitrates are utilized as rapidly as they are formed.

Ammonia can serve as a source of nitrogen for cellulose-fermenting organisms without first being converted to nitrates. Since ammonia was present in cel-

lulose-containing soils when all of the nitrates had been utilized, it appears that the ammonification process continues in the presence of cellulose.

It thus appears that none of the soil processes are inhibited by the addition of carbohydrates to the soil; this is to be expected, for the carbohydrates are not toxic to these organisms. What occurs in the presence of the cellulose is that conditions in the soil are improved for the development of microorganisms and these in turn utilize the available nitrogen for their growth, the nitrogen usually becoming a limiting factor. The growing plant appears to be quite unable to compete with the microörganisms, hence the crop failures on soils containing cellulose.

PART II

It is thought fitting to record here an experiment conducted for the purpose of obtaining some information concerning the availability, for the cellulose fermentation, of organic nitrogen such as would be present in the protein residues returned to the soil under natural conditions or obtained as cleavage products from such residues.

As this experiment was commenced before those described in part I, no information was to be had concerning the influence of the more firmly bound nitrogen on this fermentation. It was thought that protein nitrogen was rendered available more slowly than seems to be the case. For this reason also it was difficult to determine when the analyses should be made, and consequently the 91-day period of incubation was much longer than necessary. Whether a shorter period would have brought out greater differences in the degrees of fermentation in the various bottles is not certain.

The experiment was conducted in exactly the same manner as those described in part I. The substances chosen for study were: ammonium sulfate, urea, aspartic acid, asparagine, alanine, tyrosine, peptone, casein, and stable manure. These substances were added to the soil in amounts such that their available nitrogen, in the case of casein total nitrogen, was equal to the ammonia added in a 2 per cent application of stable manure. For the ammonium sulfate, peptone, casein, and manure, the nitrogen values given in table 5 were obtained by analysis; for the other compounds, these values were calculated according to formula, only the purest compounds obtainable being used. One per cent of cellulose and the amounts of nitrogenous material equivalent to the values given in the table were added to the soil, which was then brought to its optimum water content of 18 per cent. Soluble compounds were added as solutions. After 91-days incubation at 18°C. the soils were analyzed for cellulose with the results indicated in table 5. All figures are the average of two closely agreeing analyses from two soils receiving similar treatment, with the exception of the soils to which aspartic acid had been added. One of the latter had an exceedingly great mold growth and contained only 7.6 per cent of cellulose at the time of analysis.

The conclusions drawn from this experiment are: that the nitrogen present in the natural residues and returned to the soils either in the form of dead vegetation or manure is more available for the cellulose fermentation than was previously supposed, and that the accelerating influence of such compounds on this process is directly proportional to their rate of ammonification. Once the protein is hydrolyzed to the amino acid stage its nitrogen seems to be practically as available as ammonia. The rate of cellulose decomposition is dependent then, not only on the ammonia and nitrate nitrogen in the soil, but also on the rate of decomposition of the more complex nitrogen-containing compounds, as illustrated by the soils containing peptone, casein, and manure.

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THE EFFECTS OF VARIOUS METHODS OF APPLYING FERTILIZERS ON CROP YIELDS[1]

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The effects of various methods of applying fertilizers on plant growth and on certain soil conditions have been considered in another publication (1). In this paper the results of field tests will be given, showing the influence of various fertilizers, applied in different ways, on some important farm crops.

The fertilizers used included acid phosphate (16 per cent), commercial brands secured from different sources, and home mixtures prepared from nitrate of soda, acid phosphate and muriate of potash. Unless otherwise stated, the 0-12-2 and 0-12-0 used were the home mixtures, whereas the 2-12-2 was Armour's Big Crop brand.

EXPERIMENTAL

Oats and clover on Carrington loam

This experiment was carried out on the Agronomy Farm to study the two methods of applying fertilizers, drilled in the rows, *direct contact with the seed* and drilled as a separate operation to the seeding or *broadcast*. A Peoria-Union fertilizer grain drill was used. Iogren oats and Shield Brand medium red clover were seeded. The fertilizer treatments varied as shown in table 1, 200 pounds per acre of 16 per cent acid phosphate being taken as the normal application and the other fertilizers being applied to carry equivalent P_2O_5.

On all the fertilized plots the oats matured earlier than on the checks. The 2-12-2 and 0-12-2 ripened from 4 to 7 days before the 0-16-0 and from 7 to 10 days earlier than the checks. Further, all of the *drilled in row* fertilizer treatments matured 3 to 6 days earlier than the *broadcast* treatments.

From the oats yields in the table it seems evident that drilling the fertilizer *direct contact in the seed rows* was better than *broadcasting* it. Such results do not mean, however, that true broadcasting (using a limesower and discing in the fertilizer, for instance) may not prove equal or even superior to the *drilled in row* method under other conditions.

The fertilizer applications to the clover gave a good stand and crop, whereas the stand and yields on the check plots were very poor. Increased rates of

[1] Part II of a thesis presented to the faculty of the Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

fertilizer application apparently produced larger weights of hay, also, in most instances. No one of the fertilizer brands used consistently out-yielded the others, but the acid phosphate seemed to give the most economical returns.

The data from this study do not warrant a conclusion concerning the better method of applying the fertilizer to clover. The *separate operation* or *broadcast* method gave as good fertilizing benefits as the *direct contact in the seed row* method.

TABLE 1

Oats and clover fertilizer study on Carrington loam

PLOT NUMBER	FERTILIZER TREATMENTS		ACRE YIELDS OF OATS GRAIN (32 POUNDS)	INCREASES BY INTERPOLATION METHOD	ACRE YIELDS OF CLOVER	INCREASES BY INTERPOLATION METHOD
	Analysis	Application				
			bushels	*bushels*	*pounds*	*pounds*
1	None	Check	29.4		794	
2	2–12–2	Drilled in row 0.5 *N**	48.8	18.1	1,802	900
3	0–12–2	Drilled in row 0.5 *N*	52.1	20.1	2,018	1,009
4	0–16–0	Drilled in row 0.5 *N*	55.8	22.5	2,234	1,117
5	None	Check	34.7		1,225	
6	2–12–2	Drilled in row *N*	52.0	14.3	2,380	1,244
7	0–12–2	Drilled in row *N*	63.3	22.6	1,875	829
8	0–16–0	Drilled in row *N*	52.7	9.0	1,802	846
9	None	Check	46.7		866	
10	2–12–2	Broadcast 0.5 *N*	50.4	7.0	1,370	613
11	0–12–2	Broadcast 0.5 *N*	53.2	13.1	1,945	1,296
12	0–16–0	Broadcast 0.5 *N*	41.2	4.3	1,514	974
13	None	Check	33.6		432	
14†	2–12–2	Broadcast *N*	58.6	25.0	1,920	1,542
15†	0–12–2	Broadcast *N*	44.3	10.7	1,685	1,361
16	0–16–0	Broadcast *N*	41.4	7.8	1,945	1,675
17	None	Check	33.6		216	
18	0–16–0	Drilled in row 0.25 *N*	45.4	11.8	1,370	1,046
19	0–16–0	Drilled in row 2 *N*	58.1	24.5	3,320	2,888
20	2–12–2	Drilled in row 2 *N*	64.8	31.2	3,675	3,135
21	None	Check			649	

* *N* = Normal application of 200 pounds of 16 per cent acid phosphate per acre.

† Shorter plots, due to accidental double-seeding in two spots which were discarded.

Oats on O'Neill loam

Table 2 gives the outline of and crop yields in this experiment, which was conducted on a farm near Ames. Iogren oats were drilled with a Van Brunt fertilizer grain drill, and medium red clover seed was sown at the same time. The broadcast applications of the fertilizers were made with a Superior limesower and then worked into the soil by the grain drill. The acid phosphate treatments were reduced from 0-16-0 to 0-12-0 in all cases, so that the same drill or limesower set could be used as was employed in the 0-12-2 and 2-12-2 brands. For the *above* fertilizer treatments, the delivery pipes of another

drill were attached to the Van Brunt drill so that the fertilizer fell partly into the row in *direct contact with the seed* but chiefly *above the seed with soil interposed.*

Although this test failed to show very pronounced fertilizer responses, some interesting facts are indicated. Drilling the fertilizer *direct contact in the seed*

TABLE 2

Oats fertilizer study on O'Neill loam

PLOT NUMBER	FERTILIZER APPLICATION			ACRE YIELDS OF GRAIN (32 POUNDS)	ACRE INCREASES BY INTERPOLATION METHOD
	Analysis	Rate per acre	Method		
		pounds		*bushels*	*bushels*
1	Check	None		34.1	
2	2–12–2	100	Drilled in seed rows	40.9	6.6
3	2–12–2	100	Broadcast with limesower	34.1	−0.4
4	0–12–0	100	Broadcast with limesower	32.9	−1.9
5	0–12–0	100	Drilled in seed rows	38.6	3.6
6	Check	None		35.2	
7	0–12–0	400	Broadcast with limesower	37.4	1.8
8	0–12–0	200	Drilled in seed rows	44.3	8.2
9	0–12–2	200	Drilled in seed rows	43.1	6.6
10	0–12–2	400	Broadcast with limesower	40.9	3.9
11	Check	None		37.4	
12	0–12–2	100	Drilled in seed rows	43.1	5.2
13	0–12–2	100	Broadcast with limesower	37.4	−0.9
14	0–12–2	100	Drilled—separate operation—(2 inches deep)	39.7	0.9
15	0–12–2	100	Drilled in seed rows (2 inches deep)	46.5	7.3
16	Check	None		39.7	
17	0–12–2	200	Drilled *above* seed through second set delivery pipes	38.6	−1.6
18	0–12–2	200	Drilled—separate operation—(½ inch deep)	42.0	1.4
19	0–12–2	200	Drilled—separate operation—(2 inches deep)	45.4	4.3
20	0–12–2	200	Drilled—separate operation—(4 inches deep)	47.7	6.2
21	Check	None		42.0	
22	0–12–0	200	Drilled—separate operation—(4 inches deep)	52.2	9.4
23	0–12–0	200	Drilled *above* seed through second set delivery pipes	44.3	0.6
24	0–12–0	200	Drilled in seed rows (2 inches deep)	54.5	10.0
25	Check	None		45.4	
26	2–12–2	200	Drilled in seed rows (2 inches deep)	55.6	10.2
27	2–12–2	200	Drilled *above* seed through second set delivery pipes	51.1	5.7
28	2–12–2	200	Drilled—separate operation—(4 inches deep)	54.5	9.1
29	Check	None		45.4	
30	2–12–2	400	Drilled in seed rows	60.2	15.5
31	2–12–2	100	Drilled in seed rows	46.5	2.6
32	Check	None		43.1	

row with the grain drill was better than *broadcasting* with a limesower. *Broadcast* applications of 100 pounds per acre failed to give increased yields over the checks, and 400 pounds *broadcast* did not yield as much as 200 pounds drilled *direct contact.* Drilling the fertilizer as a *separate operation to the seeding* was

slightly better than *broadcasting* it with a limesower. Where the *separate operation* method was used, the depth of fertilizer application relative to the seed appeared important. Drilling the fertilizer *above* the seed through a second set of delivery pipes gave very small benefits.

Winter wheat on Carrington loam

In this experiment on the Agronomy Farm, a special 2-12-2 mixture deriving its ammonia one-half from calcium cyanamid and one-half from dried blood, was prepared for comparison with Armour's 2-12-2 Big Crop brand.

Iobred wheat was used at the rate of 1½ bushels per acre. Each plot was 1 drill strip wide and 163.5 feet long, or $\frac{1}{8}$ acre in size. The fertilizer applications were made with a Peoria-Union drill for the row methods and with a Peoria limesower for the broadcast.

From the results given in table 3, some significant facts may be noted. Four hundred pounds per acre of Cyanamid 2-12-2 fertilizer applied *direct contact in the seed row* gave an increase over the adjacent check but the increase was smaller than that given by the fertilizer applied in safer ways. Four hundred pounds per acre of the commercial 2-12-2 drilled *direct contact* also depressed the yield when compared to the same treatment applied *broadcast*. The injury was not so pronounced, however, as in the case of the fertilizer containing Cyanamid.

Two hundred pounds per acre of 0-12-2 drilled *direct contact* gave the best yields. The same amount of 0-12-0 similarly applied was almost as effective. Four hundred pounds *broadcast* was not so beneficial as 200 pounds *direct contact*. Drilling the fertilizer as a *separate operation to the seeding* gave benefits equal to those of the limesower *broadcast* method. The separate drilling failed to equal the *direct contact in the seed rows* method for moderate amounts of the non-caustic fertilizers.

Fall applications of fertilizers were more beneficial than spring applications. Splitting the fertilizer between fall and spring applications did not appear to be economical.

Applying the fertilizer 10 days in advance of seeding gave good results for the Cyanamid mixture, but was of no value for the other fertilizers studied. The depth study showed that locating fertilizers (particularly acid phosphate) shallower than the seed at planting time was not so beneficial as locating them at the same depth or slightly deeper than the seed.

Fertilizer depth study with winter wheat on Carrington loam

In this experiment on a farm near Ames, the depth factor of fertilizer location relative to the seed was studied. The fertilizer was applied with the Peoria-Union grain drill on the day of seeding, but as a *separate operation to sowing the Iobred wheat*. The plots were 530 feet long and 1 drill strip wide, or approximately $\frac{1}{12}$ acre.

TABLE 3

Winter wheat fertilizer study on Carrington loam

PLOT NUMBER	FERTILIZER TREATMENTS			ACRE YIELD OF GRAIN (60 POUNDS)	ACRE INCREASES BY INTERPOLATION METHOD
	Analysis	Application			
		Rate per acre	Method		
		pounds		*bushels*	*bushels*
1	Check	None		31.6	
2	2–12–2 (Cyanamid)	400	Broadcast wth limesower	38.0	6.3
3*	2–12–2 (Cyan.)	400	Broadcast with limesower	39.2	7.4
4*	2–12–2 (Cyan.)	400	Drilled	38.0	6.0
5	2–12–2 (Cyan.)	400	Drilled—separate operation	38.0	5.9
6	2–12–2 (Cyan.)	400	Drilled in seed rows	34.8	2.6
7	Check	None		32.3	
8*	0–12–2	400	Broadcast with limesower	37.7	5.1
9	0–12–2	400	Broadcast with limesower	39.9	7.1
10	0–12–2	200	Drilled in seed rows	44.6	11.5
11	0–12–0	200	Drilled in seed rows	43.0	9.6
12	0–12–0	400	Broadcast with limesower	39.6	5.9
13*	0–12–0	400	Broadcast with limesower	39.2	5.3
14	Check	None		34.2	
15†	2–12–2 (Commercial)	400	Broadcast with limesower	36.1	1.7
16	2–12–2 (Com.)	400	Broadcast with limesower	44.3	9.7
17	2–12–2 (Com.)	400	Drilled in seed rows	42.1	7.2
18*	2–12–2 (Com.)	400	Drilled	42.4	7.3
19	2–12–2 (Com.)	400	Drilled—separate operation	43.0	7.6
20†	2–12–2 (Com.)	400	Drilled	38.0	2.4
21	Check	None		35.8	
22†	0–12–2	400	Drilled	36.4	0.8
23	0–12–2	400	Drilled—separate operation	37.3	2.0
24	0–12–2	400	Drilled in seed rows	40.2	5.2
25	0–12–0	400	Drilled in seed rows	38.6	3.9
26†	0–12–0	400	Drilled	36.1	1.7
27	Check	None		34.2	
28	2–12–2 (Commercial)	400	Half drilled in seed rows in fall Half drilled in spring	36.7	2.5
29	2–12–2 (Com.	200	Drilled in seed rows	38.0	3.8
30	2–12–2 (Com. †0–12–2	200 200	Drilled in seed rows Drilled	37.3	3.0
31	2–12–2 (Commercial)	400	Drilled in seed rows (2 inches deep)	40.5	6.2
32	2–12–2 (Com.)	400	Drilled—separate operation (½ inch deep)	37.3	2.9
33	2–12–2 (Com.)	400	Drilled—separate operation (4 inches deep)	38.3	3.9
34	Check	None		34.5	

* Fertilizer applied 10 days before seeding.

† Fertilizer applied in the spring.

The data in table 4 show that drilling the fertilizer *direct contact in the seed rows* was superior to drilling the same fertilizer as a *separate operation to the seeding*, no matter what depth was used in the drilling. Even 100 pounds per

TABLE 4

Fertilizer depth study with winter wheat on Carrington loam

PLOT NUMBER	FERTILIZER TREATMENTS				ACRE YIELDS OF GRAIN (60 POUNDS)	ACRE INCREASES BY INTERPOLATION METHOD
	Analysis	Application				
		Rate per acre	Depth	Method		
		pounds	*inches*		*bushels*	*bushels*
1	Check	None			29.4	
2	2–12–2 (Cyanamid)	400	½	Drilled—separate operation after seeding	36.8	6.3
3	2–12–2 (Cyan.)	400	2	Drilled—separate operation	39.8	8.2
4	2–12–2 (Cyan.)	400	4	Drilled—separate operation before seeding	41.1	8.3
5	2–12–2 (Cyan.)	400	2	Drilled in seed rows	43.9	9.9
6	2–12–2 (Cyan.)	100	2	Drilled in seed rows	41.7	6.5
7	Check	None			36.4	
8	2–12–2 (Commercial)	400	½	Drilled—separate operation after seeding	37.6	2.8
9	2–12–2 (Com.)	400	2	Drilled—separate operation	38.8	5.6
10	2–12–2 (Com.)	400	2	Drilled in seed rows	41.5	9.9
11	2–12–2 (Com.)	400	4	Drilled—separate operation before seeding	37.8	7.8
12	2–12–2 (Com.)	100	2	Drilled in seed rows	37.2	8.8
13	Check	None			26.8	
14	0–12–0	400	½	Drilled—separate operation after seeding	29.4	1.2
15	0–12–0	400	2	Drilled in seed rows	37.8	8.2
16	0–12–0	400	2	Drilled—separate operation	37.0	6.0
17	0–12–0	400	4	Drilled—separate operation before seeding	35.5	3.0
18	Check	None			33.9	
19	0–12–0	100	2	Drilled in seed rows	38.8	5.5
20	0–12–2	100	2	Drilled in seed rows	40.0	7.2
21	0–12–2	400	½	Drilled—separate operation after seeding	35.6	3.4
22	0–12–2	400	2	Drilled—separate operation	36.0	4.3
23	0–12–2	400	4	Drilled—separate operation before seeding	37.2	6.1
24	0–12–2	400	2	Drilled in seed rows	38.0	7.5
25	Check	None			30.0	

acre of the fertilizers drilled *direct contact* always proved equal to or slightly better than 400 pounds of the same fertilizer applied as a *separate operation.* Little increase in yield over the 100-pound applications was found for the 400-pound treatments *direct contact.*

The conclusion appears warranted that applications of fertilizers shallower than the seed were not equal in benefits to applications on the same plane with the seed or slightly deeper.

Winter wheat on Tama silt loam

This experiment was carried out on Tama silt loam in Adair County. Table 5 gives the outline of the test and the yields obtained. Turkey Red wheat was

TABLE 5

Winter wheat fertilizer study on Tama silt loam

PLOT NUMBER	FERTILIZER TREATMENTS			ACRE YIELDS OF GRAIN (60 POUNDS)	ACRE INCREASES OF GRAIN OVER AVERAGE OF CHECKS
	Analysis	Application: Rate per acre	Application: Method		
		pounds		*bushels*	*bushels*
			Large acreage test		
1	Check	None		11.5	
2	2–12–2	200	Drilled—separate operation	15.7	3.9
3	2–12–2	200	Drilled in seed rows	21.2	9.4
4	2–12–2	200	Drilled in spring	15.1	3.3
5	Check	None		12.1	
			Small plot test		
1	Check	None		12.1	
2	2–12–2	300	Drilled in seed rows	15.7	4.5
3	2–12–2	100	Drilled in seed rows	14.5	3.3
4	2–12–2	200	Drilled in seed rows	20.6	9.4
5	2–12–2	200	Drilled half in fall, half in spring	16.3	5.1
6	2–12–2	300	Drilled, 200 pounds in fall, 100 pounds in spring	16.9	5.7
7	2–12–2 0–12–0	200 100	Drilled in seed rows in fall Drilled in spring	18.8	7.6
8	0–12–0	300	Drilled, 200 pounds in fall, 100 pounds in spring	17.6	6.4
9	0–12–0	200	Drilled, half in fall, half in spring	15.7	4.5
10	0–12–0	200	Drilled in seed rows	20.0	8.8
11	0–12–0	100	Drilled in seed rows	15.7	4.5
12	0–12–0	300	Drilled in seed rows	17.6	6.4
13	Check	None		10.3	

seeded at the rate of 1½ bushels per acre with a Moline fertilizer grain drill. The large acreage portion of the experiment consisted of 5 plots, each 98 feet wide and 660 feet long, or approximately 1½ acre in size. In the small plot test each plot was 1 drill width across the field. The complete commercial fertilizer, 2-12-2, was furnished by the American Agricultural Chemical Company.

In the large acreage test, it is evident that drilling the fertilizer *direct contact in the seed rows* was preferable to either fall or spring *separate operation* drilling. On the small plots, the 200-pound applications drilled in *direct contact* again gave the best yields. The 300-pound treatments showed depressions in yields, being scarcely better than the 100-pound applications. The combinations of fall plus spring applications failed to equal the yields with fall applications alone, even where 300 pounds applied in this way was compared to 200 pounds applied in the fall. It will be noted in both parts of this study that the best application of the fertilizer practically doubled the yields over that on the check plots.

TABLE 6

Winter wheat fertilizer study on Tama silt loam II

PLOT NUMBER	FERTILIZER TREATMENTS			ACRE YIELDS OF GRAIN (60 POUNDS)	ACRE INCREASES BY INTERPOLATION METHOD
	Analysis	Application			
		Rate per acre	Method		
		pounds		*bushels*	*bushels*
1	Check	None		10.3	
2*	2–12–2	300	Drilled	12.1	1.9
3	2–12–2	300	Drilled—separate operation (2 inches deep)	16.9	6.9
4	2–12–2	300	Drilled in seed rows (2 inches deep)	22.4	12.6
5	Check	None		9.7	
6	2–12–2	100	Drilled in seed rows (2 inches deep)	16.9	6.6
7	2–12–2	300	Drilled—separate operation (½ inch deep)	16.3	5.4
8	2–12–2	300	Drilled—separate operation (2 inches deep)	17.6	6.1
9	2–12–2	300	Drilled—separate operation (4 inches deep)	17.6	5.5
10	Check	None		12.7	
11	0–12–0	300	Drilled—separate operation (½ inch deep)	18.2	6.4
12	0–12–0	300	Drilled—separate operation (2 inches deep)	16.9	6.0
13	0–12–0	100	Drilled in seed rows (2 inches deep)	18.8	8.9
14	0–12–0	300	Drilled—separate operation (4 inches deep)	18.2	9.3
15	Check	None		7.9	
16	0–12–0	300	Drilled in seed rows (2 inches deep)	19.3	10.8
17	0–12–2	300	Drilled in seed rows (2 inches deep)	18.2	9.1
18	0–12–2	100	Drilled in seed rows (2 inches deep)	14.5	4.8
19	0–12–2	300	Drilled—separate operation (2 inches deep)	15.7	5.4
20*	0–12–2	300	Drilled	12.7	1.8
21	Check	None		11.5	

* Fertilizer applied in the spring.

Winter wheat on Tama silt loam II

This test was conducted on another farm in Adair County. Kanred wheat was seeded at the rate of 1½ bushels to the acre and drilled in with the Moline fertilizer grain drill, on plots of 1 drill width each. The 2-12-2 fertilizer was Armour's Big Crop brand.

The results given in table 6 show that drilling the fertilizer *direct contact*

in the seed rows was better than drilling it as a *separate operation* either in the fall or in the spring. Indeed, the *direct contact* plots averaged 20 bushels per acre, whereas the unfertilized checks averaged only 10.4 bushels and the spring applications averaged 12.4 bushels. The most economical treatment was the 100 pounds per acre of 0-12-0.

The depth study indicated that shallow fertilizer applications were not equal to similar ones located on the same plane with the seed or even slightly deeper.

Winter wheat on Grundy silt loam

This experiment was located in Wapello County. Since no fertilizer grain drill was available, the applications were made with a Holden, rear end-gate broadcast fertilizer and limestone distributor. It was planned to compare the two common methods of working the fertilizer into the soil after it

TABLE 7

Winter wheat fertilizer study on Grundy silt loam

PLOT NUMBER	FERTILIZER APPLICATION			ACRE YIELDS OF GRAIN (60 POUNDS)	ACRE INCREASES BY INTERPOLATION METHOD
	Analysis	Rate per acre	Method		
		pounds		*bushels*	*bushels*
1	Check	None		36.3	
2	2–12–2	400	Broadcast and disced in	46.0	10.3
3	0–12–0	400	Broadcast and disced in	38.7	3.6
4	0–12–2	400	Broadcast and disced in	43.0	8.5
5	Check	None		33.9	
6	2–12–2	400	Broadcast and harrowed in	40.6	7.2
7	0–12–0	400	Broadcast and harrowed in	35.7	2.7
8	0–12–2	400	Broadcast and harrowed in	40.0	7.5
9	Check	None		32.1	

had been applied broadcast, *discing in* versus *harrowing in.* The results of the experiment are given in table 7.

The harvest yields showed that the *discing* method gave larger yields than the *harrowing* method for all of the fertilizers tested. For light sandy soils in a rainy season, the results might be reversed, but for the average soil of the cornbelt the thorough discing in of the fertilizer appears desirable.

Corn on Carrington loam I

This experiment was carried out on the Agronomy Farm. Table 8 gives the outline with the harvest data. The *hill* fertilization was attained with the standard attachment on a John Deere No. 999 corn planter, which instead of depositing the fertilizer *in the hill*, spread the fertilizer as a strip approximately 1 to 1½ inches wide and 3 to 6 inches long at a distance of 3 inches *to the rear* of the hills.

The plots were 4 rows wide or 2 drill widths of 7 feet each. Reid's Yellow Dent was used and the seed was planted about 2½ inches deep. The broadcast fertilizer was drilled only to moisture.

In considering the harvest results, emphasis should be placed on the fact that the *rear of hill* location for the fertilizer gave 3 to 7 days earlier maturity than the *broadcast* treatments. For maximum yields and superior quality of corn, however, the *broadcast* treatments appeared the better.

TABLE 8

Corn fertilizer study on Carrington loam I

PLOT NUMBER	FERTILIZER TREATMENTS		ACRE YIELDS OF CORN (70 POUNDS)	ACRE INCREASES BY INTERPOLATION METHOD
	Analysis	Application		
			bushels	*bushels*
1	None	Check	77.1	
2	2–12–2	Broadcast 0.5 *N**	83.3	6.1
3	0–12–2	Broadcast 0.5 *N*	79.0	1.6
4	0–16–0	Broadcast 0.5 *N*	77.9	0.3
5	None	Check	77.7	
6	2–12–2	Broadcast *N*	83.3	7.9
7	0–12–2	Broadcast *N*	83.9	10.8
8	0–16–0	Broadcast *N*	72.9	2.1
9	None	Check	68.5	
10	2–12–2	Hill 0.5 *N*	78.0	8.9
11	0–12–2	Hill 0.5 *N*	79.9	10.2
12	0–16–0	Hill 0.5 *N*	73.0	2.7
13	None	Check	70.9	
14	2–12–2	Hill *N*	72.3	1.7
15	0–12–2	Hill *N*	69.9	−0.5
16	0–16–0	Hill *N*	67.6	−2.5
17	None	Check	69.8	

* *N* = normal application of 200 pounds of 16 per cent acid phosphate per acre, taken as basis for applications.

Corn on Carrington loam II

This experiment was carried out the following year on the same land used in the preceding test. A slight change in the order of arranging the fertilizer treatments was made, the 0-12-0 mixture being placed between the 2-12-2 and the 0-12-2, to determine whether potash had any influence upon the corn yields. The acid phosphate, or 0-16-0, used in the first year was diluted to an 0-12-0 mixture. The number of plots was increased for a more complete test.

Table 9 gives the outline of the experiment with the harvest yields. The corn was planted with a John Deere No. 999 fertilizer corn planter. The plots were 4 corn rows wide, or 14 feet, and about 100 hills long, so that $\frac{1}{10}$-acre plots might be harvested. The *broadcast* fertilizer treatments were made with the Peoria-Union fertilizer grain drill after the corn had been planted. The *hill* applications were made with a new attachment designed by the writer and

connected to the seed shanks. It consisted of two regular attachments joined by iron braces and operated by the checkwire simultaneously with the seed valves. It applied the fertilizer partly in *direct contact* and partly along *both sides* of the hills as desired, and although it was not perfect because the poor furrow openers used increased the draft considerably, it served to give some interesting comparisons.

From the harvest data it appears that the potash applications with acid phosphate gave benefits in all cases. The 2-12-2 fertilizer showed up better

TABLE 9

Corn fertilizer study on Carrington loam II

PLOT NUMBER	FERTILIZER APPLICATIONS			ACRE YIELDS OF CORN (70 POUNDS)	ACRE INCREASES BY INTERPOLATION METHOD
	Analysis	Rate per acre	Method		
		pounds		*bushels*	*bushels*
1	Check	None		67.6	
2	2–12–2	134	Broadcast—grain drill	79.6	9.3
3	0–12–0	134	Broadcast—grain drill	80.0	7.0
4	0–12–2	134	Broadcast—grain drill	86.4	10.7
5	Check	None		78.3	
6	2–12–2	267	Broadcast—grain drill	82.4	3.1
7	0–12–0	267	Broadcast—grain drill	86.9	6.6
8	0–12–2	267	Broadcast—grain drill	88.6	7.3
9	Check	None		82.3	
10	2–12–2	134	Both sides of hill	83.3	4.6
11	0–12–0	134	Both sides of hill	75.4	0.4
12	0–12–2	134	Both sides of hill	83.4	12.1
13	Check	None		67.6	
14	2–12–2	267	Both sides of hill	74.0	7.2
15	0–12–0	267	Both sides of hill	62.7	−3.2
16	0–12–2	267	Both sides of hill	71.9	6.9
17	0–12–2	267	Broadcast—grain drill	69.2	5.1
18	Check	None		63.2	
19	0–12–2	134	Both sides of hill	74.8	13.2
20	0–12–2	134	Broadcast—grain drill	67.0	6.9
21	0–12–2	134	Rear of hill	68.8	10.3
22	Check	None		57.0	

in the *hill* plots than in the *broadcast* plots when compared to the adjacent 0-12-0 plots, but failed to equal the results secured with the 0-12-2 treatments in most cases. With the latter mixture the *hill* method out-yielded the *broadcast* method. Hill fertilization upon *both sides* by the new attachment was better than *to the rear* with the standard attachment. The economy of smaller applications located at the hill is shown also in this test. For early maturity the hill method of corn fertilization again proved itself superior to broadcasting.

Corn on Carrington loam III

The outline of this test is presented in table 10, with the harvest data. It was carried out on a farm near Ames. Kelly's Improved seed corn was used.

TABLE 10

Corn fertilizer study on Carrington loam III

PLOT NUMBER	FERTILIZER APPLICATIONS			ACRE YIELDS OF CORN (70 POUNDS)	ACRE INCREASES BY INTERPOLATION METHOD
	Analysis	Rate per acre	Method		
		pounds		*bushels*	*bushels*
1	Check	None		48.9	
2	2–12–2	100	Both sides of hill	50.3	1.7
3	2–12–2	100	One side of hill	47.4	−1.0
4	2–12–2	100	Continuous in row, falling upon "deflectors" at bottom of delivery pipes	48.6	0.5
5	2–12–2	100	Continuous in row, direct contact	45.7	−2.2
6	Check	None		47.6	
7	2–12–2	200	200 pounds 0–12–2 broadcast (limesower) 21.3 pounds $NaNO_3$ both sides of hill	12.4a 35.7b 48.1	0.6
8	2–12–2	200	150 pounds broadcast (lime sower) and 50 pounds both sides of hill	51.9	4.5
9	2–12–2	200	Both sides of hill	52.1	4.8
10	2–12–2	200	Broadcast (limesower)	50.1	3.0
11	Check	None		47.0	
12	2–12–2	150	150 pounds 0–12–2 broadcast (grain drill) 16 pounds $NaNO_3$ both of sides hill	17.1a 25.6b 42.7	−4.3
13	2–12–2	150	Broadcast (grain drill)	45.7	−1.3
14	2–12–2	150	100 pounds broadcast (grain drill) and 50 pounds both sides of hill	46.6	−0.3
15	2–12–2	300	200 pounds broadcast (grain drill) and 100 pounds both sides of hill	44.0	−2.9
16	Check	None		46.9	
17	2–12–2	300	Broadcast (grain drill)	49.9	3.1
18	2–12–2	100	Both sides of hill	51.3	4.6
19	2–12–2	100	Rear of hill	47.6	1.0
20	2–12–2	300	Broadcast (limesower)	47.7	1.2
21	Check	None		46.4	
22	2–12–2	100	Broadcast (limesower)	49.1	2.8
23	2–12–2	100	One side of hill	51.6	5.4
24	2–12–2	100	Rear of hill	48.4	2.3
25	2–12–2	100	Broadcast (grain drill)	48.4	2.4
26	Check	None		45.9	

a = 2 inside rows.
b = 2 outside rows.

The plots were $\frac{1}{10}$ acre in size, 4 rows wide and 89 hills long. Plot 23 was intended to be a *both sides of the hill* treatment but as one of the rods operating the fertilizer dump valves on the right seed shank broke, the treatment was

TABLE 11

Potato fertilizer study on Carrington fine sandy loam

PLOT NUMBER	METHODS OF FERTILIZER APPLICATION (1600 POUNDS PER ACRE OF 3-12-4)	ACRE YIELDS	ACRE INCREASES BY INTERPOLATION METHOD
		bushels	*bushels*
1	Check—unfertilized	155.0	
2	*Sides lower plane*—narrow spread	217.0	61.7
3	¼ fertilizer *below* seed and ¾ at *sides same plane*—narrow spread	201.0	45.4
4	¾ fertilizer in *made-up ridge* ahead of planting and ¼ at *sides same plane*—narrow spread	187.5	31.6
5	½ fertilizer in *made-up ridge* ahead of planting and ½ at *sides same plane*—narrow spread	199.5	43.3
6	Check—unfertilized	156.5	
7	*Made-up ridge* ahead of planting	194.5	39.7
8	*Sides same plane*—wide spread	192.5	39.4
9	*Sides same plane*—narrow spread	205.0	53.6
10	*Made-up ridge* ahead of planting	172.0	22.3
11	Check—unfertilized	148.0	
12	*Sides same plane*—wide spread	189.0	40.1
13	*Sides lower plane*—narrow spread	215.0	65.2
14	*Sides same plane*—narrow spread	199.5	48.9
15	Check—unfertilized	151.5	
16	¼ fertilizer *below* seed and ¾ at *sides same plane*—narrow spread	198.0	47.8
17	*Above* seed with soil interposed, wide spread	165.0	16.2
18	*Above* seed with soil interposed, narrow spread	161.5	14.1
19	Check—unfertilized	146.0	
20	¼ fertilizer *above* seed and ¾ at *sides same plane*—narrow spread	182.5	38.9
21	*Direct contact* in row (1600 pounds per acre)	117.0	−24.2
22	*Mixed with soil in row* (1600 pounds per acre)	131.0	−7.8
23	*Direct contact in row* (400 pounds per acre)	149.5	13.1
24	Check—unfertilized	134.0	
	(0–16–0 applied in this part of the study)		
25	*Direct contact* in row (400 pounds per acre)	137.5	1.2
26	*Sides same plane*, narrow spread (400 pounds per acre)	168.5	29.9
27	Check-unfertilized	141.0	
28	*Made-up ridge* ahead of planting (400 pounds per acre)	172.0	31.3
29	*Above* seed with soil interposed, wide spread (400 pounds per acre)	146.0	5.7
30	¼ fertilizer *above* seed and ¾ at *sides same plane*, narrow spread (400 pounds per acre)	170.5	30.6
31	Check—unfertilized	139.5	
32	*Sides same plane*—narrow spread (1200 pounds per acre)	179.0	37.8
33	*Sides same plane*—narrow spread (1600 pounds per acre)	180.5	37.6
34	*Sides same plane*—narrow spread (800 pounds per acre)	179.0	34.4
35	*Sides same plane*—narrow spread (2400 pounds per acre)	191.0	44.7
36	Check—unfertilized	148.0	

one side of hill. The *side-hill* attachment on the right seed shank was set in too closely, so that a part of the fertilizer fell in *direct contact* with the corn kernels in the hill. This location proved quite injurious to the germination where the nitrate of soda was checked at the hills in plots 7 and 12. The outer rows of these plots planted with the left shank were of average stand.

The yields obtained in this experiment are small, but the comparisons are quite definite. The conclusion seems warranted that the *hill* methods of fertilizer application were better than the *broadcast* methods. The *sides of hill* location also seemed superior to the *rear of hill* location. No conclusions can be drawn from the results with the *split* applications.

Potatoes on Carrington fine sandy loam

This experiment was carried out on a farm near Clear Lake, Iowa. Two rows of Rural New Yorker seed were planted in each plot with a 3-foot interval between the rows. The rows were each 64 rods long, thus making plots of approximately $\frac{1}{7}$ acre. A new Iron Age Automatic potato planter was used.

Table 11 gives the outline of the test with the harvest results. The fertilizers used were Armour's 3-12-4 Big Crop brand, and 16 per cent acid phosphate. All of the *made-up ridge* fertilizer applications were secured by using the planter to apply the fertilizer as a separate operation with wide set of the delivery pipes, then shutting off the fertilizer attachment and retracing the ridges to plant the seed. The *mixed with the soil in the row* condition was secured with the same adjustment as in the *direct contact* method, except that a log-chain was looped back and forth from the opening discs and allowed to drag in the seed row just ahead of the seed delivery chute.

It appears from the data in the table, that applications of large amounts of high-analysis, readily-soluble fertilizers *direct contact in the row with the seed* reduced the yields. Applied in this manner, 1600 pounds per acre of fertilizer actually decreased the yield by about 30 bushels below the average of all of the checks. Mixing the same amount of fertilizer with the soil in the row tended to minimize the injury but did not eliminate it.

Applications of small amounts of fertilizer *direct contact in the rows with the seed* sometimes gave increased yields, but the results cannot be said to equal those secured with better methods of application.

The best method of fertilizer application seemed to be the *sides lower plane* location. Increases of over 60 bushels per acre were secured by this method.

The *made-up ridge* plots failed to indicate any definite facts, and no conclusions can be drawn regarding the practice of *splitting* the fertilizer applications.

CONCLUSIONS

These experiments must be repeated upon numerous soil types and over a series of years before definite conclusions can be drawn and a satisfactory

fertilizer practice worked out for each of the various crops. The results reported, however, indicate some very worthwhile suggestions.

In fertilizing cereals like oats and wheat, the use of a combination fertilizer-grain drill is recommended. Moderate applications of non-caustic fertilizers gave the most economical returns by this *direct contact in the seed rows* method. As much as 400 pounds per acre of 16 per cent acid phosphate or 300 pounds of commercial 2-12-2 fertilizer were drilled with the seed without appreciable injury to germination and hence resulting decreased yields.

Larger applications than these or the distribution of fertilizers containing caustic ingredients like Cyanamid, should be made separately from the seeding. The best yields with the Cyanamid came from broadcasting it ten days in advance of the seeding. For large applications of non-caustic fertilizers, the best method appeared to be a splitting of the total application between the *drilled direct contact in the seed rows* method and the *broadcast* method.

These tests showed no advantage in spring applications, either for the entire fertilizer application or for a part of it. The drilling of the fertilizer as a *separate operation to the seeding*, no matter what the depth or the time period before the seeding, was not equal to the *direct contact* method, provided of course moderate amounts of the non-caustic fertilizers were used. The use of a second set of delivery pipes for distributing the fertilizer *above* the seed-row was found to safeguard the germination, but failed to give benefits warranting their adoption. Where *broadcast* applications of fertilizers were made with a limesower or by other means upon the seedbed surface, the best results were secured when the fertilizer was worked into the soil by thorough discing.

In the fertilization of corn, the *hill* methods appeared equal to or even superior to the *broadcast* method if the fertilizer was not delivered *direct contact into the hills with* the seed. Earlier maturity was secured with the hill method.

Comparisons of the two hill methods—the *rear of hill* with the *sides of hill*—indicated that the latter was preferable. It cannot give the *direct contact in the hill* location as the *rear of hill* method often does (because of a deranged adjustment of the fertilizer attachment) and hence the *sides* method is recommended over the *rear* method for preventing injury to seed germination.

A satisfactory design of attachment is needed to guarantee the *sides* location of the fertilizer for all machines sowing seeds in wide rows or in hills, such as corn, cotton, beets, and potatoes. In fact, the *sides lower plane* location for the fertilizer gave the best yields of potatoes in these Iowa tests.

REFERENCE

(1) Coe, D. G. 1926. The effects of various methods of applying fertilizers on crops and on certain soil conditions. *In* Soil Sci., v. 21, p. 7-21.

A COMPARATIVE STUDY OF THE BACTERIAL FLORA OF WINDBLOWN SOIL: I. ARROYO BANK SOIL, TUCSON, ARIZONA

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INTRODUCTION

Although a considerable amount of work has been done upon the bacteria in soil, no one apparently has made a comparative study of the number and types of bacteria in ecologically equivalent types of soil in different regions. It was, therefore, with this aim in view that the series of studies, of which this is the first, was started. The type of soil selected for the study was arid, windblown soil, such as is found in the dunes along the coast and around Lake Michigan, and in the dry regions in the interior of the continent.

In order to facilitate the handling of such a comprehensive piece of work, the approach was made through the study of certain types of aerobic bacteria that grow in ordinary culture media. Should the study of these forms prove to be of value, the work probably will be extended to those forms which require special culture conditions.[1]

METHODS AND MATERIALS

Collection of samples

The soil was collected with a trowel at three places 10 to 15 feet apart, from depths of 6, 12, and 24 inches. It was thoroughly mixed in quart jars, which had previously been scrubbed out with the same soil, one jar being used for each depth.

Study of the soil

Water content. At the time of collection the water content of the soil was determined. The soil was not oven-dried, because of lack of temperature control, but was exposed, with repeated stirrings, to the Arizona sunshine until the weight was constant.

Water capacity. The water capacity was tested some time after the soil was collected. As the quantity of soil was limited, 10 cc. of distilled water was allowed

[1] The work included in the present report was carried on at the Desert Laboratory of the Carnegie Institution of Washington. The author wishes, therefore, to express her deep appreciation of the courtesy and assistance extended by Director MacDougal, and the other members of the laboratory. The photographs of Pantana Wash were taken by Mrs. Strickland and Mr. Godfrey Sykes of the Desert Laboratory.

to percolate through a weighed amount of the oven-dried sample, the percolate was collected, and the capacity was calculated. This was checked by weighing.

Size of soil particles. A rough analysis was made by passing the soil through a series of sieves and bolting cloth with apertures having diameters of 2 mm., 1 mm., 0.5 mm., and approximately 0.124 mm. respectively.

Combustible material. Oven-dried soil was weighed on an asbestos plate of known weight. It was then heated and stirred until there was no further evidence of combustion. After this it was heated a few minutes longer and reweighed. The asbestos plate was also reweighed and the loss calculated.

Reaction of the soil. The importance of the soil reaction became evident when, after the soil had stood almost two years, the test was made. As the soil had been kept perfectly dry during this time, no great chemical change seemed possible. The test was made by adding 1 gm. of soil to 5 cc. of distilled water, as advocated by Joseph and Martin (31) and used by Atkins (4) and others. To the mixture of soil and water was added 10 drops of indicator, made according to Gillespie's directions (25). The mixture was shaken repeatedly for about half an hour. As the soil was light and settled rather quickly, it was not decanted nor centrifuged. After the soil had settled the liquid was matched with the indicator chart of Clark (11).

Water-soluble salts. Considering the chemical composition of the soil, the water-soluble salts, particularly the chlorides, carbonates, sulfates and nitrates, are probably the most significant from the standpoint of bacterial flora. An analysis on this basis was made, therefore, according to the method given in Official and Tentative Methods of Analysis (2).[2]

Plating

Due to an unavoidable delay, the soil was plated 36 hours after collection. Dilutions of 1:10,000; 1:100,000; and 1:1,000,000 were made by shaking the required amount of soil in the proper amount of distilled water, for two or three minutes, and allowing the heavy particles to settle. According to Hiltner and Störmer (28) adequate shaking removes the bacteria from soil particles and a transfer of portions of the soil, as advocated by Wyant (59) is unnecessary. Three, or six, plates were poured from each dilution for each sample, in nutrose agar, made according to a modification of the formula suggested by Waksman and Fred (57). Five-tenths per cent nutrose was used, the sugar was omitted and the salts were supplied by tap water.

Counting

The counting was done with the naked eye, supplemented by a lens whenever threre was any doubt regarding the colonies.

[2] The analysis was made by Miss Miriam Dice, under the direction of Dr. Mary A. Griggs, Associate Professor of Chemistry, Wellesley College, to whom the writer presents her sincere thanks.

Isolation of forms

From the original plates 84 colonies were fished and streaked on nutrose agar. From 4 of these 2 were separated, making a total of 88 streaks. Actinomycetes were avoided in this selection whenever their nature was evident. These cultures were placed in cold storage in Tucson for the summer, and shipped to Wellesley in August.[3] Here those that were still alive were transferred to nutrient agar (made from 1.5 per cent Difco Dehydrated Nutrient Agar) and kept as stock cultures, for further study.

Study of morphology

Slides were made from these cultures and stained, first by Gram's method, as modified by Sterling [Hiss and Zinsser (29, p. 103)]. When this staining did not show the form clearly, Loeffler's methylene blue, or Ziehl's carbol fuchsin, was used. On account of pressure of work, the motility of the forms escaped attention until many had died.

Culture reactions

Glucose gelatin. Glucose gelatin was made by adding 10 gm. of glucose and 0.5 cc. each of a 1.6 per cent solution of brom-cresol-purple and cresol-red to a liter of gelatin, made from Difco Dehydrated Nutrient Gelatin. This formula is that suggested by the Committee of the Society of American Bacteriologists (42, p. A 10). With this medium the reaction of the bacteria to glucose and their ability to digest gelatin, and, in the case of the non-liquefiers, their relation to oxygen, could be observed.

Sucrose broth. Sucrose broth was made by adding sucrose and indicators, as in the case of glucose gelatin, to Difco Dehydrated Nutrient Broth, and tubing it in Dunham's fermentation tubes.

Purple milk. Purple milk, made from Difco Dehydrated Purple Milk, was used to test fermentation of lactose, production of rennin, and digestion of casein.

Nitrate broth. Nitrate broth was made by adding 0.02 per cent potassium nitrate to Difco Dehydrated Nutrient Broth. Griess' sulfanilic acid test, given by Fred (23) was used for testing the presence of nitrites.

EXPERIMENTS AND RESULTS

Study of the soil

Situation. The place selected for the collection of the soil was upon the southeast bank of Pantana Wash, at the left of the bridge by which the Tanque Verde Road crosses the wash, a short distance from Tucson. The situation was on top of the high bank and, although in time of flood the wash contains

[3] Through the courtesy of Mr. Godfrey Sykes.

water, the author was assured that the place selected had probably had no water on it, except rain, for at least four years. (See plate 1.) In this connection the following data regarding the annual rainfall were given in an article by MacDougal (41) and supplemented by information from the Desert Laboratory.

Rainfall at the Desert Laboratory

	inches
1918	10.37
1919	19.66
1920	10.51
1921	15.93
1922	10.53
1923	12.61
Average	13.27

This, according to Lipman (38) would be classified as a "truly arid soil." The surface was bare, except for scattered shrubs (see plate 2) and the soil was quite dry and moving with the wind. The collection was made on April 18, 1923.

TABLE 1

Water content of soil

DEPTH	WEIGHT			WATER
	At start	After drying	Loss	
inches	*gm.*	*gm.*	*gm.*	*per cent of dry soil*
6	200	198.10	1.90	0.95
12	200	194.70	5.30	2.65
24	200	195.15	4.85	2.43

TABLE 2

Water capacity of soil

DEPTH	AMOUNTS USED		PERCOLATED WATER	RETAINED WATER	WATER
	Soil	Water			
inches	*gm.*	*cc.*	*cc.*	*cc.*	*per cent of dry soil*
6	10.00	10	7.2	2.80	28
12	8.75	10	7.8	2.20	25
24	10.00	10	7.0	3.00	30

TABLE 3

Combustible material in the soil

DEPTH	BEFORE BURNING	AFTER BURNING	TOTAL LOSS	LOSS OF PLATE	LOSS OF SOIL	WATER
inches	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent of dry soil*
6	50	49.2	0.8	0.1	0.7	1.4
12	50	48.7	1.3	0.2	1.1	2.2
24	50	46.0	4.0	3.0	1.0	2.0

Water content. The water content, tested April 18 and 19, 1923, is shown in table 1.

Water capacity. The water capacity, tested March 25, 1925, is shown in table 2.

Combustible material. The amount of combustible material, determined June 16, 1924, is shown in table 3.

Size of soil particles. Table 4 shows the sizes of soil particles in 100 gm. of dry soil tested on June 16, 1924.

TABLE 4

Various sized particles in 100 gm. of dry soil

DEPTH	>2 MM.	1–2 MM.	0.5–1 MM.	TOTAL	0.124–0.5 MM.	<0.124 MM.	TOTAL
inches	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
6	0.05	0.20	3.40	3.65	56.70	39.70	96.40
12	0.60	1.20	4.40	6.20	38.20	55.40	93.60
24	0.05	0.50	2.30	2.85	40.10	57.10	97.20

TABLE 5

Summary of the physical characters of the soil

DEPTH	WATER CONTENT	WATER CAPACITY	PARTICLES BELOW 0.5 MM.	COMBUSTIBLE MATERIAL	RELATIVE WATER CONTENT*	OPTIMUM WATER CONTENT†
inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	0.95	28	96.4	1.4	3.4	19.6
12	2.65	25	93.6	2.2	10.4	17.5
24	2.43	30	97.2	2.0	8.1	21.0

* By relative water content is meant the ratio $\frac{\text{water content}}{\text{water capacity}}$.

† Optimum water content is taken to be 70 per cent of capacity.

TABLE 6

Hydrogen-ion concentration of the soil

DEPTH	THYMOL BLUE	CRESOL RED
inches	*pH*	*pH*
6	9.0	8.8
12	8.6	8.6
24	8.8	9.0

From the comparison given in table 5 it appears that, although the differences are slight, at 12 inches this particular soil is a little coarser than at 6 or 24 inches, with a correspondingly lower water capacity. It also contains a little more combustible material and has a little higher actual and relative water content.

Reaction of the soil. The hydrogen-ion concentration of the soil, tested February 28, 1925, is shown in table 6.

As this was so close to the range limit of Cresol red, the experiment was again repeated, this time with Thymol blue. This second test agreed exactly with the first one made with this indicator and was accepted as correct.

Water-soluble salts. The analysis was made in June, 1925, two years after the soil was collected. As the soil had been kept perfectly dry and tightly closed, probably the only change which might have taken place is a loss of carbon dioxide, which would probably have been lost in equal amount, if at all, from all three samples. The figures given in table 7 are the results of duplicate tests, which were either identical or checked rather closely. In the latter case the average is given. The percentages are for dry soil.

TABLE 7

Water-soluble salts in the soil

DEPTH	TOTAL SALTS	Cl	CO_3	SO_4	OTHER SALTS
inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	0.0595	0.0070	0.0090	0.001±	0.0505
12	0.0732	0.0151	0.0204	0.0018	0.0359
24	0.0284	0.0070	0.0087	Trace	0.0197

TABLE 8

Average numbers of organisms per gram of fresh soil

DEPTH	DILUTION*	NUMBER OF PLATES	AFTER 6 DAYS	AFTER 10 DAYS	AVERAGE 10 DAYS
inches					
6	1:10,000	6	238,000	352,000	
	1:100,000	6	320,000	450,000	401,000
12	1:10,000	3	1,560,000	1,930,000	
	1:100,000	6	1,367,000	1,867,000	1,898,500
24	1:10,000	6	425,000	783,000	
	1:100,000	6	650,000	1,050,000	916,500

* The number of colonies was so small on the 1:1,000,000 dilution plates that the count was not considered.

The figures under "other salts" in table 7 were obtained by subtraction from total salts and, therefore, contain the various cations of the anions given, plus the nitrates and traces of anything else soluble. If the cations were eliminated the 12-inch sample would be reduced more than the 6-inch or 24-inch samples, and this would make the difference in the nitrate content still greater.

As the 12-inch layer, although containing the most carbonates, has the lowest hydroxyl-ion concentration, it would seem that the carbonate cannot be the sodium salt, since, according to Atkins (3) very little of this salt is needed to raise the alkalinity to pH 10. He considers that the hydrogen-ion concentration is dynamic, that it varies with temperature, water content and other factors, and that it is a waste of time to try to measure it with great care and accuracy.

The numbers of organisms in the soil

The results shown in table 8 take into consideration only the fungi, actinomycetes, yeasts, and bacteria which grow under the conditions of the experiment. The plates were poured on April 20, 1923 and were counted after 6 and 10 days incubation at room temperature.

The numbers in table 8 were calculated per gram of fresh soil, according to the method proposed by Waksman (55). Reduced to number per gram of dry soil the figures become, for 6 inches 405,050 per gm.; for 12 inches, 1,957,216 per gm.; and for 24 inches, 935,204 per gm.

One set of plates for each depth was selected as showing the most varied assortment of colonies. A count was made and recorded in table 9. In this

TABLE 9

The numbers of colonies of various forms found on plates

DEPTH	DILUTION	NUMBER OF PLATES	TOTAL NUMBER	ACTINOMYCETES		FUNGI		YEASTS, BACTERIA	
inches					*per cent*		*per cent*		*per cent*
6	1:10,000	6	200	97	48.50	1	0.50	102	51.00
12	1:100,000	5	99	47	47.47	1	1.01	51	51.51
24	1:10,000	6	470	259	55.01	4	0.85	207	44.04
All.....		17	769	403	52.40	6	0.77	306	46.81

TABLE 10

Colored bacteria, or yeasts, on the plates

DEPTH	TOTAL NUMBER OF COLONIES	WHITE	YELLOW	ORANGE	RED	FLUORESCENT	BROWN
inches		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	102	85.29	7.84	0.98	1.96	0.98	2.94
12	51	90.19	5.88	0.00	0.00	0.00	3.92
24	207	89.37	5.79	0.00	0.96	1.93	1.93
All.....	360	88.33	6.39	0.28	1.11	1.39	2.50

table and in subsequent discussions "bacteria" is used to indicate the lower forms and does not include the actinomycetes.

Considering those colonies without the recognizable character of fungi or actinomycetes, as bacteria or yeasts, a count was made of the various colors appearing on the plates. The results are shown in table 10.

As is usual, white forms predominate, but the striking point in the above table is the general scarcity of colored forms on plates which had been selected for the greatest variation in forms.

It should be noted, in regard to the counts given in tables 9 and 10, that some of the colonies classified as bacteria, or yeasts, may have been actinomycetes in an early stage of development, before their characteristic appearance was evi-

dent. That this is probably true is shown by the fact that some of the colonies that were streaked for stock cultures, proved later to be actinomycetes. In this connection it is interesting to consider the relative numbers of actinomycetes, bacteria, and yeasts, and the distribution of colors in the stock cultures as shown in tables 11 and 12. It will be understood that, in fishing colonies for pure cultures, the effort was made to secure as many different kinds as possible, hence the proportions of colored forms do not agree with those found in plate counts, where many colonies were of the same kind.

TABLE 11

Numbers of various forms fished for pure cultures

DEPTH	TOTAL NUMBER	ACTINOMYCETES	YEASTS	BACTERIA	UNDETERMINED*
inches					
6	26	2	1	21	2
12	27	3	1	20	3
24	35	1	4	13	17
All.....	88	6	6	54	22

* These cultures died before their morphology was studied.

TABLE 12

Distribution of color in forms fished for pure cultures

DEPTH	TOTAL NUMBER*	WHITE, GREY OR CREAM		YELLOW OR ORANGE		RED OR PINK		BROWN	
inches			*per cent*		*per cent*		*per cent*		*per cent*
6	24	18	75.0	5	20.8	1	4.2	0	0
12	26	20	76.9	5	19.2	1	3.8	0	0
24	23	13	56.5	8	34.8	1	4.3	1	4.3
All....	73	51	69.9	18	24.7	3	4.1	1	1.4

* Some of the streaks did not grow, and no color could be determined

The study of pure cultures

The original cultures isolated in 1923 were transferred at intervals and careful observations were made to insure, as far as possible, purity of cultures and healthy growth. The total number of forms under observation was 88. Cultures died out as time went on, others were lost by contamination, in others two forms were separated, and still others proved, upon study, to be actinomycetes or yeasts and were not carried further. At the end of the work 38 were growing normally, 38 had died out or been lost by contamination, 6 were classified as yeasts and 6 as actinomycetes. In tables 11 and 12 may be seen the numbers of the various forms and the distribution of colors in the bacteria and yeasts.

Morphology. Cultures 10 days old were stained by Gram's method and studied. Interpretation was difficult in some cases and more slides were made

at intervals, during the course of the work, and stained in various ways. Table 13 is a summary of the results.

From table 13 it will be seen that a little over a half (57.4 per cent) were non-spore-bearing forms, whereas 42.6 per cent formed spores. About half of the spore-formers were Gram-positive, and made up the bulk of those taking this stain; 10 out of 13 coccus (or coccoid) forms, 8 out of 11 short rods and 5 out of 7 non-spore-bearing long rods were Gram-negative.

Culture reactions. All culture reactions were run in duplicate and repeated whenever the two tubes did not agree. A summary of the production of acid

TABLE 13

Summary of the morphology of bacteria in pure cultures

DEPTH	TOTAL NUMBER	COCCUS OR COCCOID	SMALL RODS	LONG RODS		GRAM'S STAIN	
				Spores	None	Negative	Positive
inches		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	21	33.30	23.80	33.30	9.50	81.00	19.00
12	20	15.00	15.00	55.00	15.00	50.00	50.00
24	13	23.10	23.10	38.40	15.40	61.50	38.50
All.....	54	24.10	20.40	42.60	12.9	64.80	35.20

TABLE 14

Summary of culture reactions at the end of 2 weeks

DEPTH	BACTERIA CAUSING ACID FERMENTATION OF SUGARS						BACTERIA DIGESTING				BACTERIA REDUCING NITRATES TO NITRITES	
	Glucose		Sucrose		Lactose		Gelatin		Casein			
inches		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*
6	5	25.0	1	5.6	0	0	14	70.0	9	50.0	5	27.8
12	9	47.4	8	47.1	0	0	13	72.2	13	76.4	8	47.1
24	4	33.3	4	36.4	1?	9.0?	10	71.4	4	36.4	2	18.2
All.....	18	35.3	13	28.3	1?	2.20?	37	72.5	26	56.5	15	32.6

in sugars, the digestion of gelatin and casein and the reduction of nitrates, after 2 weeks incubation at room temperature, is given in table 14.

a. Glucose gelatin was inoculated on January 5, 1924, and observed after 2, 4, 7 and 14 days. No gas was observed at any time.

b. Dunham's fermentation tubes of *sucrose broth* were inoculated on July 8 and 16, 1924, and observed after 2, 4, 8 and 15 days. No gas was observed at any time.

c. Purple milk was inoculated on July 16, 1924, and observed after 2, 7 and 14 days. The forms acted upon lactose more slowly than upon glucose or sucrose and there was no observable change in 2 days. There was no coagulation at any time.

d. Nitrate broth was inoculated on July 16, 1924, and tested for nitrites at the end of 1 and 2 weeks.

Of the liquefiers 22 showed distinct liquefaction on the fourth day and 15 in one to two weeks. It was not found possible to group the forms into all the

types suggested by Conn (13, 15, 19). The actinomycetes formed a clearly defined group. The cocci, none of which fermented sugars were about evenly divided among rapid, slow, and non-liquefiers. The proportion of rapid to slow to non-liquefiers in the small rods and coccoid forms was 6:3:6; in the long spore-bearing rods, 12:4:7; and in the long non-spore-bearing rods, 2:4:1.

From table 14 it appears that bacteria living at 12 inches from the surface in this soil are, in general, more active in their chemical processes than those living at 6 or 24 inches, since the largest percentages of acid formers, of casein and gelatin digesters, and of nitrate reducers were found at this depth.

It is to be regretted that so many forms died during the process of the work, which was unavoidably extended over 2 years. Had it been possible to concentrate the work the numbers would have been larger and the results more convincing.

DISCUSSION

Although the significance of this work can be brought out only after the data for other regions are complete, it is advisable to bring together in brief form the results obtained for this type of soil in comparison with those obtained by other investigators.

A review of the literature relating to bacterial counts at various depths, brings out a rather uniformly consistent opinion that the greatest number is in the upper 6 to 8 inches, regardless of the character of the soil studied (5, 10, 28, 39, 53, 54). In a number of instances (8, 5, 32, 53) 4 inches gave the maximum count. From this upper layer of great abundance the numbers in general, decrease with depth. Fränkel (22) is sometimes quoted as having found the highest number at ½ meter. A careful study of his tables, however, shows that the majority of them give no data between the surface and ½ meter. Those instances, therefore, in which the maximum number was at this point, have no significance except that the maximum was not at the surface, as it was in a large proportion of cases. Reimers (47) also used, in general, 1-meter and ½-meter intervals, and his "surface" seems to refer to the whole layer of loose surface soil, which varied considerably in thickness.

In the Tucson soil, of the three layers studied, the largest number of bacteria occurred in the 12 inch stratum, the next largest in the 24 inch layer, and the smallest number at 6 inches from the surface. At 12 inches the average number was 1,957,216 per gram of dry soil and at 24 inches 935,204. A comparison of these figures with the results of Chester (10)—75,000 to 124,000 at 12 inches and 4,000 at 24 inches—suggests that in arid soil the whole curve representing numbers to depth, shifts downward. The fact that bacteria penetrate to greater depths in arid soils than in those of humid regions, is discussed by Lipman in several articles (37, 38, 39).

The work of Cutler, et al. (20) indicates that there are two maxima for the bacterial count in the year, one at the end of June and the other at the end of November. If this be true for all regions, the soil was collected at Tucson

about half way between a maximum and a minimum, and should, therefore, be a fair average. As daily platings were not made, the fluctuations described by Cutler were not noted, or taken into account.

In considering the factors which might be responsible for the distribution of bacteria in the Tucson soil, the most obvious is lack of water. As has been indicated, the average annual rainfall for the year in which the collection was made, and for the five years previous was 13.27 inches. This, combined with the data in tables 1 and 2, shows that the soil is exceedingly dry.

The effect of moisture upon the numbers of bacteria in soil has been considered by a number of investigators. The problem of the bacterial count in arid soil, however, involves more than a consideration of the effect of soil moisture upon the number of bacteria in experimental pots. Pot cultures, to which certain amounts of water are added, or which are allowed to dry out, cannot duplicate exactly the conditions in the field. That drying out of such soil should give a decrease in the number of bacteria, seems reasonable (46), but the work of Waksman (53) indicates that the variation in number does not always agree with the variations in moisture content. There is also considerable disagreement in the numbers of bacteria in soil sampled in the open. Sampling under various conditions led Waksman (56) to conclude that a long dry spell decreases the number of bacteria, whereas a wet spell increases it. Prescott (45) found a decrease in number of bacteria in Egyptian soil after the summer fallow period, during which the soil bakes. Cutler, et al. (20), on the other hand, found, by daily platings, that there were great and unaccountable variations in the numbers, which did not coincide with variations in soil moisture, rainfall, soil temperature, nor seasonal temperature. This great variation is confirmed by Engberding (21), whose curves of bacterial counts show great irregularities, but the greater peaks and hollows of which agree roughly with the curve of monthly precipitation. Russell (49) also noted the same phenomenon of great and irregular variation, but attributed it to variations in protozoal content. It is quite conceivable that a bacterial flora adapted to moist conditions might be much more sensitive to variations in soil moisture than one which had adapted itself to dry conditions. A careful study of this problem would probably yield interesting results.

Although the soil at Tucson was loose and sandy in appearance, a rough analysis showed that a large proportion was of fine material, a characteristic which will probably be of importance in future comparisons. Notwithstanding this fine character, the water capacity was low, partly, no doubt, because of the small amount of organic material. The reaction of the soil was very alkaline (pH 8.6 to 9) and the upper layer had the highest pH value. This test was made about two years after the collection of the samples, which had, nevertheless, been kept tightly closed and dry during the interval. According to several observers (1, 7, 48), soil, especially that on the alkaline side of neutrality, becomes more acid upon storage and drying. The results submitted in table 6, therefore, are probably more acid than they would have been

with fresh soil. Arrhenius (1) states that 14 days standing has no effect upon the reaction, but he cautions against long storage. The data for wind-blown soils have been gathered from coastal dunes, and will be discussed in the second part of the work.

Stewart (52) has made many chemical analyses of Utah soils, some with, and others without, "alkali." By comparing his tables with table 7 many interesting points may be noted. Except for certain "surface" collections, his first sample was taken at a depth of 12 inches, and the others at 1-foot intervals. In his tables the percentages are given for definite salts rather than for the atomic groups as in the analysis of the Tucson soil, consequently only approximate comparisons can be made. In all his analyses, except for one surface—non-alkali—soil, the percentages of total salts are much higher than in the Tucson soil, and since the chlorides, carbonates, and nitrates are fairly comparable, the difference lies chiefly in the sulfate content. The percentages of total salts and of individual salts, except nitrates, increased downward in the Utah soils, whereas in the Tucson soil the highest percentages of these salts was at 12 inches. The tendency for the nitrates to accumulate in the upper layer agrees with Stewart's analyses.

The fact that the 12 inch layer contained the most soluble salts, except nitrates, and also had the highest bacterial count, suggests that in these concentrations, chlorides, carbonates and sulfates may be stimulating. There has been a considerable amount of work upon the effect of salts upon the development and activities of bacteria, but since the investigators have used different amounts of the salts, different criteria for evidence of effect, and different conditions under which the experiments were conducted, it is exceedingly difficult to correlate the results. Lipman (34) found that Na_2CO_3 stimulated ammonifying bacteria, but that neither NaCl nor Na_2SO_4 had that action. Greaves (26) found that the common soil alkalies, NaCl, $CaCl_2$ Na_2SO_4 and $NaNO_3$ were toxic to these bacteria; that there was no stimulating action in any concentration of NaCl, $Ca(NO_3)_2$, KCl, K_2SO_4, $Mg(NO_3)_2$ or Na_2SO_4, but that the other salts used stimulated this process at some concentration. Fred and Hart (24) report that $CaSO_4$ stimulates ammonification. With respect to nitrifying bacteria, Lipman (35) found that very weak solutions of NaCl and Na_2SO_4 were stimulating, whereas Na_2CO_3 was toxic at the highest dilution used (0.025 per cent). Brown and Hitchcock (6) agree, in general, with this work, but found the toxic points to differ from those of Lipman, and include Na_2CO_3 among the salts stimulating in dilute solutions. For the nitrogen-fixing organisms Lipman (36) found that there was no stimulation by any of the three salts studied, in the dilutions used. Chester (9) found that $NaNO_3$ and KCl used in the proportion of 1 part of salt to 5,000 parts of soil, or 0.02 per cent, were without appreciable effect on the bacterial development in the soil.

In the Tucson soil the dilutions of salts were below the toxic points found by the aforementioned investigators. Using 0.2 *M* concentration, Hohn and

Sherman (30) found that nitrates, as well as chlorides and sulfates, accelerated the growth of *B. coli*. In the 6 inch layer of Tucson soil the amount of nitrates was much less than that used by these investigators, although it was somewhat greater than the amounts in the 12 inch and 24 inch layers. It is possible that this slight excess, associated with other unfavorable conditions, such as relatively low water and organic content and high alkalinity, helped to inhibit the growth of bacteria, whereas the greater porosity, higher water and organic content, lower alkalinity, and higher content of salts with stimulating action, favored the growth of organisms in the 12 inch layer.

According to Hilgard (27) the salts in arid soil are carried down a few feet by the scant water supply, and accumulate there, whence they rise, by capillarity, with the evaporating water. Stewart (52) notes that in some sections, where the soil is well drained, there is a marked lack of salts, because they have been carried down to lower land. This seems to be the probable explanation for the low salt content of the soil at the edge of Pantana Wash, much of the salts having been carried down into the wash by percolating water. The 12 inch layer is probably the stratum in which the descending and ascending waters cause a slight accumulation. Whether the accumulation of nitrates in the 6 inch layer is due to the physico-chemical nature of the salts, or to greater nitrification in that layer, cannot be determined without extended experimentation.

The variation in kinds of bacteria is exceedingly important. No attempt has been made to identify specific organisms, but merely to ascertain the relative proportion of types. Yeasts, actinomycetes, and bacteria have been distinguished and the latter grouped morphologically and according to a limited number of physiological reactions.

It is impossible to give plate counts of yeasts unless every colony is examined, because it is impossible superficially to distinguish them from bacteria. Also it is impossible to know how many of the colonies given in plate counts by other writers were yeasts.

The actinomycetes are more easily distinguished, although in the early stages, and in the case of small, deep colonies, it is impossible to distinguish them from bacteria or yeasts. Lipman and Waynick (40) state that there is a decrease in the number of bacteria when a soil is transferred from a humid to an arid climate, and also a decrease in nitrification and nitrogen-fixation, but not in cellulose destruction. Waksman and Heukelekian (58) have shown that cellulose destruction may be carried on by fungi, actinomycetes, and bacteria. Arid soil appears to be unfavorable to the growth of fungi, hence the cellulose destruction in it must be due predominantly to actinomycetes and bacteria. Since the work of T. L. Martin (43) showed that actinomycetes were affected by the addition of green manure more than were bacteria, it is possible that the actinomycetes are more active in the destruction of cellulose than the lower forms. It is possible, therefore, that the statement of Lipman and Waynick suggests, at least, that there may be more actinomycetes in arid soil than in

other situations. Waksman (53) studied the distribution of fungi, actinomycetes, and bacteria in various soils (textures not given) and concluded that although bacteria decreased downward and fungi almost disappeared below 12 inches, the actinomycetes remained constant between 12 inches and 30 inches, hence they increased in proportion to the other groups in the lower layers. A comparison of table 9 with his results, shows a fairly close agreement in the numbers of fungi and actinomycetes at the deeper layers, but a considerable difference for all counts at 12 inches and for the bacteria at the lower level. At 12 inches the Tucson soil contained 4 to 6 times as many actinomycetes, only one-sixth to one-seventh as many fungi, and about five-eighths as many bacteria as the soils studied by Waksman. Although the work of W. H. Martin (44) upon potato scab showed an increase of the disease in dry alkali soils, Sanford (50) concluded that lack of moisture alone was responsible for the disease. His work was with heavily infected soils, consequently lack of infection did not indicate lack of organisms. The work seems to indicate that dry, and possibly alkaline, soils are favorable for actinomycetes. Conn (14, 18) found that sod contained a larger number of actinomycetes than cultivated soil—38 per cent and 20 per cent, respectively—and thought that the difference was not due to lack of aeration in sod, but possibly to the presence of plant roots. In other reports (12, 19) upon various soils, he gives the percentages of these forms as anywhere from 12 to 50. Hiltner and Störmer (28) give as an example, a plate counting showing over 25 per cent of actinomycetes. Krainsky (33) placed the number of these forms in dune sand between those for sandy woods and beech woods, but gave no figures.

Conn's suggestion, that the function of actinomycetes in the soil is the decomposition of plant roots, does not exclude the disintegration of cellulose. Both functions would imply a correlation between the number of these forms and the quantity of vegetable matter in the soil. On the other hand, the number of actinomycetes is larger in Tucson soil than in any soil reported, except in the general statement by Conn noted above, that they compose 12 to 50 per cent of the soil flora. The percentage for the 24 inch layer exceeds even this estimate by about 5 per cent. (See table 9.) It is, therefore, difficult to account for the high percentage of these organisms in the Arizona soil studied.

That chromogenesis is confined largely to yellow and fluorescent forms (15) is only partly confirmed by the present investigation. In general, there was a great lack of color; even on nutrose agar over 88 per cent of the colonies were white. Although yellow was the most prevalent color, the percentage of yellow colonies was very low. The fact that brown forms were next in abundance is interesting, but its significance is not known. Red, orange, and fluorescent forms were scarce and no purple, blue, or black forms were found.

In several publications Conn (12, 13, 15, 16, 17) discussed the types of bacteria in soils, and in 1917 (19) he summarized his previous work and classified the types into several groups. Of these the non-spore-bearing forms are the

most abundant, and the spore-bearers few and unimportant. The percentage of the latter group is given as 5 to 10. Of the non-spore-formers, he found rapidly liquefying short rods of the *Pseudomonas fluorescens* type to be less than 10 per cent, and short rods or cocci, slow or non-liquefiers, which do not ferment sugars, to be from 40 to 75 per cent. Many of these grow poorly on laboratory media. He noted the absence, or presence in such small numbers as to be overlooked, of forms that ferment sugars with gas, of non-liquefying acid formers, and of non-spore-bearers growing anaerobically in sugar broths. No mention is made of the reaction to Gram's stain. Skinner (51) also found aerobic spore-formers to be about 5 to 10 per cent of the total count in various soils.

A consideration of table 13 will show a totally different distribution of types in the Arizona soil from those discussed above. Of the cultures studied morphologically, 57.4 per cent were non-spore-bearing, and of these, about 58 per cent were rods. The forms were distributed rather unevenly in the three layers. The cocci were most abundant in the upper layer, and least at 12 inches. The spore-formers were most abundant at 12 inches, and least at 6 inches. The short rods were least abundant at 12 inches, and in about equal numbers at 6 inches and 24 inches. The non-spore-forming long rods were least abundant at 6 inches, and about equally abundant at 12 inches and 24 inches. Gram negative forms predominated, especially at 6 inches (81 per cent). At 12 inches the forms were evenly divided between positives and negatives, and at 24 inches there were about twice as many negatives as positives. At 12 inches were found the largest percentages of acid formers, gelatin and casein digesters, and nitrate reducers. That this is not due wholly to the spore-formers is shown by the fact that about 50 per cent of the digestion of gelatin and casein was due to other forms. At this depth the spore-formers, however, are the active sugar fermenters and nitrate reducers.

The total lack of gas in sugar fermentation, and the probable total lack[4] of lactose fermenters, agrees with the results of Conn, noted above. Approxima tely 35 per cent of the total number of forms studied formed acid in glucose, ands28 per cent in sucrose. The fact that one-third of the glucose and 2 of the sucroye fermenters were non-liquefiers, is at variance with his findings. Approximatela 57 pe r cent of the forms studied digested casein, 72.5 per cent digested gelatin, and 33 per cent reduced nitrates. Of the liquefiers, about 60 per cent were rapid, i.e., produced a fair amount of liquefaction in 4 days. The digestion of gelatin was the most active process carried on by the organisms studied. Protein digestion presupposes organic matter and it is difficult to account for such active digesters in soil with so small an amount of organic material. On the other hand, this activity would insure that the small amount of such material would quickly and efficiently be made available.

[4] The single form that caused a slightly acid reaction in milk may have been a contamination, or it may have been one of the "few" which Conn did not find.

SUMMARY

1. In this study only those bacteria are considered which grow aerobically on ordinary culture media.

2. The soil under consideration was collected at depths of 6 inches, 12 inches and 24 inches, on the edge of Pantana wash at the side of the Tanque Verde Road, Tucson, Arizona.

3. The average annual rainfall for 6 years, including the one in which the soil was collected, was 13.27 inches. This would make it, according to Lipman, a "truly arid soil."

4. The 12-inch layer had the greatest water content, both in actual amount and in relation to the water capacity. It was a little coarser, and had a larger amount of combustible material, and of soluble chlorides, carbonates and sulfates, than the other layers. It was more nearly neutral (pH 8.6) than the other samples. These characteristics were correlated with the highest bacterial count.

5. That the 6-inch layer was the least favorable for bacteria, was shown by the lowest count, and was probably due to the fact that this layer was the driest and had the least combustible material, the highest hydroxyl-ion concentration (pH 9.0), and the largest amount of nitrates of the three.

6. The numbers of bacteria per gram of fresh soil for the depths were as follows: 6 inches, 401,000; 12 inches, 1,898,500; 24 inches, 916,500.

7. Of the total number of colonies for all depths, 52.4 per cent were actinomycetes, 0.77 per cent were filamentous fungi and 46.81 per cent were yeasts and bacteria. The actinomycetes were actually and proportionally more numerous at 24 inches than at 6 or 12 inches.

8. Of the colonies which were not recognized as actinomycetes, and were therefore classified as bacteria and yeasts, 88.33 per cent were white; 6.39 per cent, yellow; 0.28 per cent, orange; 1.11 per cent, red; 1.39 per cent, fluorescent; and 2.5 per cent, brown.

9. Pure cultures were isolated from the plates and studied morphologically and culturally.

10. Morphologically, 24.1 per cent were coccus, or coccoid, 20.4 per cent were short, non-spore-bearing rods, 13.0 per cent were long non-spore-bearing rods, 42.6 per cent were long spore-bearing rods, and 64.8 per cent were Gram negative.

11. Culturally, 35.3 per cent fermented glucose; 28.3 per cent fermented sucrose; and only one form fermented lactose; 70.4 per cent digested gelatin and 56.5 per cent digested casein; 32.6 per cent reduced nitrates to nitrites.

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PLATE 1

FIG. 1. View of the Pantana Wash and the Tanque Verde Bridge. Taken from the bottom of the wash and looking toward the bank on top of which the soil samples were collected.

FIG. 2. Nearer view of the bank.

FIG. 1

FIG. 2

PLATE 2

FIG. 1. General view of the situation in which the soil samples were collected.
FIG. 2. A nearer view of the surface at the place of collection.

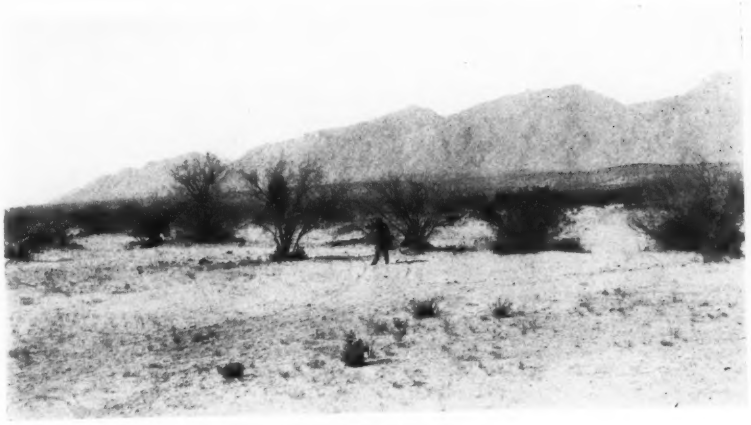

FIG. 1

FIG. 2